

**Population bottlenecks and the risk of parasitic and
microbiological infections in the endangered saddleback
(*Philesturnus carunculatus*) and South Island robin (*Petroica
a. australis*)**

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This thesis is dedicated to the memory of my brother,

Russell C. Hale

(03/08/1973 – 24/12/1997)

“There are two choices: To live or to exist.

One is a life lived to the fullest with a great passion, believing there are no limits, no boundaries, no conformities. But it is not without risk; sometimes ending too soon, a fleeting moment in time - but a great moment. However the other, though it may be long and enduring, carries an even greater risk: to have never lived at all.

Dare to live”.

- K. A. Hale

ABSTRACT

Severe population bottlenecks and the small size of many remnant habitats may render many bird populations prone to extinction from disease outbreaks. Bottlenecks may increase inbreeding which in turn may result in a low diversity of resistance and an immune system that is impaired or defective. Thus, bottlenecked populations may be less immunocompetent and more vulnerable to microbiological and parasitic perturbations. Few studies have assessed the effect of bottlenecks on the immunocompetence of birds. In this study, I used twelve saddleback (*Philesturnus carunculatus*) and two New Zealand robin (*Petroica a. australis*) populations, to determine if the severe bottlenecks reduce the immunocompetence of birds. When I experimentally challenged the immune system of two robin populations I found that despite the two populations having similar parasite loads, robins from the severely bottlenecked Motuara Island population exhibited a significantly lower T-cell mediated immune response than the source population (Nukuwaiata Island) suggesting that birds passing through severe population bottlenecks have a compromised immunocompetence. In the saddleback, severe bottlenecks, as well as high population densities and small island size, lead to individuals exhibiting higher stress levels and feather mite loads and lower immune function, as was evident by lower lymphocyte counts. I did not find levels of fluctuating asymmetry of saddlebacks to be directly influenced by bottleneck size. However, I did find that individuals with higher levels of fluctuating asymmetry had higher loads of hippoboscids and lower loads of coccidia suggesting a possible trade-off between growth and immune function. In contrast to previous studies looking at behavioural secondary sexual traits, I found no effect of founder number on the size of wattles in saddleback. I did however demonstrate that wattle size reflected the level

of immune function in females as well as males, suggesting that females play a far greater role in offspring fitness than has been appreciated in traditional theories of sexual selection.

Overall, my results indicate that severe bottlenecks can lead to reductions in immunocompetence in the resulting populations, especially in those populations that pass through the most severe bottlenecks. Based on the evidence from my thesis, I recommend conservation managers should aim to use at least 90 individuals to found new populations in order to reduce the deleterious effects of bottlenecks on immune function. If the costs of population bottlenecks and inbreeding are to be avoided, conservationists must adequately address the role of genetic factors in susceptibility to disease, and work towards minimising the risk of severe population bottlenecks in the management of endangered birds

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Chapter 1

General Introduction

1.1 Disease in wild populations

All species are faced by continual challenges from their environment. However, global scale anthropogenic changes such as pollution, habitat fragmentation, climate change, and human population expansion, have amplified the role of many environmental stressors as natural regulating factors, to the point where they have become major threats to the continued existence of vast numbers of species (Deem *et al.* 2001; Cleaveland *et al.* 2002; Frankham 2005).

Infectious disease is a major emerging threat which conservation managers are currently ill equipped to manage or prevent (Woodroffe 1999; Cleaveland *et al.* 2002). Historically, diseases of wildlife populations have only received attention when they have threatened agriculture or human health (Daszak *et al.* 2000), for example, salmonella transmission from birds to humans (Kapperud & Rosef 1983; Alley *et al.* 2002) and tuberculosis from possums to domestic stock (Roberts 1996; Kao & Roberts 1999). Today disease is recognised as a major factor contributing to the extinction of numerous wild populations, the most well known example being the widespread elimination of Hawaiian land birds following the introduction of avian malaria and avian pox (Warner 1968; van Riper *et al.* 1986). Despite numerous cases of infectious disease outbreaks in the wild, disease remains a conservation problem that is still too frequently overlooked, with disease management only being implemented when outbreaks have caused sudden declines in endangered populations (Cleaveland *et al.* 2002). Such “crisis management” is often poorly executed with little learnt in the process. Thus despite a change in attitude towards the role of disease in species decline, the focus remains largely on the outcome rather than prevention and risk management.

1.2 Inbreeding and the immune system

Recently, theoretical and experimental research suggests immunodeficiency, the inefficient functioning of the immune system, may be a common consequence of reduced genetic diversity and inbreeding (Potts *et al.* 1994; Reid *et al.* 2003; Hawley *et al.* 2005; Whiteman *et al.* 2006; Hale & Briskie 2006), with inbreeding being linked to a number of disease outbreaks in wild populations (e.g. O'Brien *et al.* 1985; Thorne & Williams 1988; Kretzmann *et al.* 1997; Saccheri *et al.* 1998). Inbreeding, defined as the mating of individuals that are more closely related than by chance alone, results in an increase in homozygosity (Frankham 1995) and has been shown to result in a number of fitness costs, such as reductions in fecundity, offspring size, growth, survivorship and developmental instability; collectively termed inbreeding depression. In any outbred population, a genetically determined differences in disease resistance or susceptibility between individuals is common. Genetically determined diversity of the immune system is the major cause for difference in resistance to diseases of infectious origin in chickens (Zekarias *et al.* 2002). Few studies have assessed the effect of inbreeding on the immunocompetence of wild birds but it is widely speculated that loss of genetic diversity and inbreeding may result in a low diversity of resistance and an immune system that is impaired or defective resulting in populations which are less immunocompetent and thus more vulnerable to disease outbreaks and high parasite burdens.

1.3 Conservation management of the New Zealand avifauna

The New Zealand avifauna has one of the highest levels of endemism in the world as well as one of the highest extinction rates (Craig *et al.* 2000). Human settlement of New

Zealand, and the subsequent reduction in forest habitat and introduction of mammals, has resulted in the elimination of many of New Zealand's native bird species. Small, largely isolated remnant sub-populations are a major feature of the current endemic bird populations (Craig 1991). Since 1960, conservation management in New Zealand has focused on using species translocations as a key management tool. This has involved eradicating vertebrate pests from offshore islands to create predator free sanctuaries to which endangered species can be shifted in order to secure their survival (Pierre 1999). The number of individuals used in translocations has been highly variable, involving as few as five birds to as many as 386 birds being transferred to islands ranging in size from 4 ha to 3000 ha (Craig 1991). Consequently, most populations on offshore islands have been through severe bottlenecks and are likely to be highly inbred. Some species have been through several sequential genetic bottlenecks, as they are transferred from one island to the next, potentially causing a serial reduction in genetic diversity. Little consideration has been given to the genetic implications of this management strategy, perhaps because of the perception that the New Zealand bird life is less susceptible to inbreeding depression due to repeated inbreeding purging deleterious alleles. However, recent studies suggest purging, particularly in small endangered populations, is not a reliable method of eliminating the risk of extinction due to inbreeding (Frankham *et al.* 2001). Jamieson *et al.* (2005) have reviewed studies of inbreeding in New Zealand and have found clear and compelling evidence that inbreeding depression does indeed exist in the New Zealand birdlife. However, there are no studies that have investigated the relationship between inbreeding and disease susceptibility in the New Zealand avifauna, and thus the risks of disease to New Zealand wildlife remains largely unknown.

1.4 The saddleback and New Zealand robin

The saddlebacks, along with the kokako (*Callaeas cinerea*) and extinct huia (*Heteralocha acutirostris*), comprise the unique endemic New Zealand wattle-bird family Callaeidae.

Both male and female saddlebacks have jet-black plumage with a highly conspicuous chestnut-coloured saddle on the back and fleshy orange wattles. This cavity nesting species is a poor flier, preferring to bound from perch to perch, and foraging mainly on the forest floor (Heather & Robertson 2000). There are two subspecies of saddleback; North Island (*Philesturnus carunculatus rufusater*) and South Island (*P. c. carunculatus*). At the time of European settlement, saddlebacks were present in forests throughout North, South and Stewart Islands and many surrounding offshore islands (Heather & Robertson 2000). The decline in saddlebacks began in the mid 19th century following extensive habitat reduction and the introduction of mammalian predators. Eventually both subspecies became extinct on the mainland. The South Island saddleback remained only on Big South Cape, Pukeweka and Solomon islands (part of the Stewart Island group), whilst the North Island saddleback was restricted to Hen Island. In the early 1960s, the accidental introduction of ship rats (*Rattus rattus*) to Big South Cape Island resulted in the rapid decline and near extinction of the South Island saddleback. In 1964, only 36 South Island saddlebacks remained and these birds were successfully translocated to Kaimohu and Big Islands. Further releases have taken place since then (Rasch & McClelland 1993) and currently approximately 1200 South Island saddlebacks range across 15 small islands, all descended from the 36 rescued from Big South Cape Island. Similarly, 6000 North Island saddlebacks

now range across 12 islands, all descended from a remnant population of 500 individuals on Hen Island (Hooson & Jamieson 2003).

The South Island robin (*Petroica a. australis*), a medium-sized (35g) ground feeding passerine, was once widespread across the South Island but, like the saddlebacks, it too has suffered decline in range and density since human settlement. The current distribution of the South Island robin is patchy on the mainland but they are locally abundant on numerous predator-free offshore islands (Heather & Robertson 2000). Only two populations of the South Island robin are included in this thesis, Motuara and Nukuwaiata islands, both located in the Marlborough Sounds of the South Island. In 1973 five individuals were translocated from Nukuwaiata Island to nearby Motuara Island to test methods for the translocation of the critically endangered Chatham Island black robin (*P. traverse*), of which only five individuals remained. The Motuara robin population expanded rapidly and now exceeds 600 individuals (Byrne 1999; Mackintosh & Briskie 2005). Thus these two populations provide an ideal model system to study the fitness consequences of a severe bottleneck by comparing the source population (or pre-bottleneck populations) on Nukuwaiata Island with its daughter or (post-bottleneck population) on Motuara Island.

1.5 Motuara Island disease outbreak

In March 1994, 26 saddlebacks were translocated from Jacky Lee and North islands and released on the Motuara Island (59 ha), in the Marlborough Sounds of the South Island. This island was selected for the release of South Island saddlebacks because the bird was believed to have been historically resident in the area and the island was free of introduced predators due to the eradication of Polynesian rats (*R. exulans*) in 1993. By 2001, the

population of saddlebacks had reached over 100 birds and all suitable habitat on the island appeared to be occupied (pers. obs.). Thus, the population was likely to be near the carrying capacity of the island (a pattern probably quite common with native birds on predator-free islands). To expand this population further, in March 2002 nine saddlebacks were translocated from Motuara Island to Long Island and further translocations were planned. However, a preliminary survey of the Motuara Island population in March 2002 revealed a catastrophic decline to only approximately 50 birds. Examination of surviving individuals revealed these birds to be infested with hippoboscids flies (pers. obs.). The family Hippoboscidae, commonly referred to as louse flies, contains about 200 species, 75% of which occur on birds. Infestation of more than four flies is rare on small birds (Hutson 1984), however the average number of flies on the Motuara Island saddlebacks was greater than 35 suggesting this population was under stress and that the high levels of infestation might be related to high population density and/or inbreeding depression. Some other individuals also exhibited symptoms of illness, skin lesions, and two autopsied corpses revealed they suffered from acute systemic coccidiosis. Whether the survivors of such population crashes can form a long-term viable population is unknown, but it is critical at this time to establish whether such parasites are widespread and whether the susceptibility of endangered birds to parasites and disease on offshore islands is linked to the increased frequency of inbreeding in island populations and/or to the effects of high population density.

1.6 Aim of thesis

The risk to New Zealand wildlife from disease is unknown at this stage, but potentially serious considering the large number of small, isolated populations of endangered species that have gone through severe bottlenecks. Do more severely bottlenecked populations exhibit lower levels of immunocompetence? Do populations founded by fewer individuals have higher parasite loads? Do more severely bottlenecked populations exhibit higher levels of stress? Is there a relationship between population density and disease susceptibility? Is there a relationship between population bottlenecks and the expression of a sexually selected trait? Can susceptible populations be detected before the effects of disease and inbreeding result in irreversible deleterious effects? These are some of the key questions I focus on in this thesis and I present a series of studies investigating immune function and parasite loads in populations of saddleback and the New Zealand robin. I use both species as models for understanding the environmental and genetic factors that may influence the levels of these two variables. The main chapters of this thesis (Two, Three, Four and Five) have been written as self-contained manuscripts for submission to scientific journals and hence there is a degree of cross-referencing and repetition which was unavoidable. The chapters are laid out in the format required by current scientific journals with abstract, introduction, methods, results, discussion and reference sections. Chapter 2, authored by myself and my supervisor, Dr. Jim Briskie, has been published in the international journal *Animal Conservation* (Volume 10, pages 2-13).

The main aim of my thesis is to draw attention to the significance of disease in the fitness of endangered wild populations of birds and the negative effects that inbreeding and habitat restriction has on the health of such populations. I begin in Chapter 2 by describing

an experiment in which I challenged the T-cell mediated immune systems of two robin populations, a founder population and a highly inbred translocated population, to investigate the effect of bottleneck size on immune function and determine if a more inbred population is less immunocompetent than one with comparatively higher levels of genetic diversity. In Chapter 3 I follow the recovery of the Motuara Island saddlebacks from the population crash described above and investigate potential factors such as high population density on the risk of disease outbreak and other fitness measures. I use a number of blood parameters to assess both levels of immune function and stress levels in the population as the population increases to above its former density. In Chapter 4, comparing 12 populations of saddlebacks, I use two hypothesised measures of stress, fluctuating asymmetry and haematological stress indices (H/L ratio), to investigate the effect of bottleneck size and island size on health, immune function and stress levels. This includes a number of morphological measurements and analysis of parasite loads, both external and internal, and white blood cell measures. Finally in Chapter 5, I take an evolutionary approach and look at the effect of population bottlenecks on the expression of a secondary sexual trait expressed by both male and female saddlebacks. The thesis concludes with a general discussion, drawing together the main findings of the thesis and brings the focus back, to some extent, to New Zealand, by focusing on current conservation strategies and potential future research that would benefit conservation management outcomes.

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Chapter 2

Decreased immunocompetence in a severely bottlenecked population of an endemic New Zealand bird*

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2.1 Abstract

Inbreeding resulting from severe population bottlenecks may impair an individual's immune system and render it more susceptible to disease. Although a reduced immune response could threaten the survival of highly endangered species, few studies have assessed the effect of population bottlenecks on immunocompetence. I compared counts of leukocytes and external, blood and gastrointestinal parasite loads in two populations of the endemic New Zealand robin (*Petroica australis*) to assess the immunocompetence of birds in a severely bottlenecked population relative to its more genetically diverse source population. Despite similar parasite loads in both populations, robins in the severely bottlenecked population showed lower counts of both total leukocyte and total lymphocytes numbers. When the immune system was experimentally challenged using the phytohaemagglutinin (PHA) skin test, robins in the severely bottlenecked population exhibited a significantly lower immune response than the source population, suggesting that birds passing through a severe bottleneck have a compromised immunocompetence. My results confirm that severe bottlenecks reduce the immune response of birds and highlight the need to avoid severe bottlenecks in recovery programs of endangered species.

2.2 Introduction

A bottleneck occurs when a population drops to a low number (Frankham *et al.* 2002). Even if such a population recovers, severe bottlenecks may increase inbreeding as survivors are forced to mate with relatives (Keller 1998). Inbreeding caused by a bottleneck may render a population more vulnerable to environmental stress, disease and extinction, as reduced genetic variation in inbred individuals could lead to their immune systems being less adaptable or defective due to the loss of specific resistance alleles (O'Brien *et al.* 1985; Thorne & Williams 1988; Spielman *et al.* 2004; Swinnerton *et al.* 2005). Reduced genetic diversity may also limit the ability of a population to adapt to new pests, climatic changes, habitat changes and introduced or co-evolving parasites (Altizer *et al.* 2001). It has been suggested that bottlenecked populations may be particularly at risk of disease outbreaks and are more prone to collapse (O'Brien & Evermann 1988; Frankham 1995; Zekarias *et al.* 2002). For example, birds in a song sparrow (*Melospiza melodia*) population that survived a severe population bottleneck on Mandarte Island were a non-random subset of the pre-crash population (Keller *et al.* 1994). Those individuals that survived were comparatively outbred compared to the individuals that died (Keller *et al.* 1994). Although bottlenecks and inbreeding may not be the only factors in determining how a population responds to disease, it may dictate which individuals survive a disease outbreak. However, few studies have experimentally tested the relationship between severe population bottleneck size and reduced immunocompetence (Reid *et al.* 2003; Hawley *et al.* 2005).

Many species around the world are currently experiencing severe population declines due to habitat loss, excessive hunting and the introduction of exotic predators. Conservation management in many endangered species has thus focused on translocating

individuals to safer or more suitable areas. For example, in New Zealand translocation to predator-free islands has been used to save at least a dozen species of endangered birds, although in most cases the number of individuals translocated is often quite small (average about 30 birds) and sometimes as few as four individuals (Craig *et al.* 2000). Likewise, translocations in other parts of the world for conservation purposes average about 75 individuals but can be as low as 30 individuals (Griffith *et al.* 1989). Such small founder populations are often quite successful in establishing new populations (e.g. Swinnerton *et al.* 2005; Taylor *et al.* 2005); however, little consideration has been given to the genetic implications of this management strategy. The rapid speed at which new pathogens can be spread around the world (Friend *et al.* 2001; Dobson & Foufopoulos 2001) could consequently threaten many endangered species if passing through a severe bottleneck reduced their ability to mount an immune response.

In this study I test the effect of population bottlenecks on immunocompetence using two closed island populations of the New Zealand robin (*Petroica australis*): an ancestral source population on one island and a new population founded 33 years ago by translocating five individuals to a second island. Thus, unlike many critically endangered species, I was able to evaluate the immune response of birds both before and after passing through a severe population bottleneck. I used surveys of parasite loads and leukocyte profiles to measure immune response to current pathogens, and then experimentally challenged birds with the phytohaemagglutinin skin test to estimate the strength of their cell-mediated immune system. My hypothesis is that individuals in a population that has gone through a severe bottleneck will have reduced immunocompetence and could be more vulnerable to future potential outbreaks of diseases and parasites.

2.3 Materials and methods

2.3.1 Study populations

The New Zealand robin is a medium-sized passerine (35g) that feeds on invertebrates on the forest floor (Heather & Robertson 2000). Robins were once widespread across New Zealand and have declined dramatically in range and density since human settlement, but survive on several predator-free offshore islands. My experiment was carried out on two such islands: Motuara Island (41°5'S 174°16'E) and Nukuwaiata Island (40°53'S 174°4'E), in the Marlborough Sounds of the South Island. In 1973 five robins were transferred from Nukuwaiata Island to nearby Motuara Island where robins were once present but disappeared when the island was cleared for farming. This transfer proved successful (the vegetation had also recovered in the interim) and the population now exceeds 600 individuals (Byrne 1999). However, recent studies show high hatching failure, lower clutch size and fewer clutches per year, suggesting that the Motuara Island robin population is suffering from inbreeding depression (Mackintosh & Briskie 2005). Genetic analyses confirm that the Motuara population has a significantly lower genetic variation than its source population on Nukuwaiata Island (Arder *et al.* 1997; Miller & Lambert 2004). Robins can be readily sexed by plumage and size differences (Heather & Robertson 2000) and I analysed my data for each sex separately. Hatch-year birds (< 1 year) can also be distinguished from adult birds by plumage differences. As parasite loads and the development of the immune system may differ with age (e.g. young birds have not been exposed to as many potential pathogens as adults), only adult robins were used in my study.

2.3.2 Parasite loads

During January-February 2005 (post-breeding autumn period), and July-August 2005 (pre-breeding spring period), I caught adult robins using a Potter trap baited with mealworm larva (*Tenebrio* sp.). All birds were colour banded for identification and an estimate of feather mite density was obtained for each individual by examining the primary feathers of the left wing. Feather mite density was given a category score from 0 - 5: 0 = no feather-mites; 1 = 0-10; 2 = 10-100; 3 = 100-1000; 4 = 1000-10000 and 5 = 10000+ feather-mites. The number of hippoboscids flies (*Ornithomya* spp. and *Ornithoica* spp.) seen on or flying off the bird were also counted. Hippoboscids flies are obligate blood-feeding ectoparasites and are known to be vectors of blood borne diseases (Hutson 1984). Faecal samples were collected for analysis for gastrointestinal nematodes and the parasitic protozoan coccidia. During the autumn faecal samples were collected from the holding bags shortly after capture; however, because I found diurnal variation in the shedding of coccidian oocysts, faecal samples in the spring were collected over the full 6-h holding period of the PHA experiment using trays in the holding cages (see below). Faecal egg counts to estimate coccidia and nematode burdens were estimated using standard faecal flotation methods carried out by a commercial laboratory (New Zealand Veterinary Pathology Ltd, Hamilton, New Zealand). Sporulation of the coccidia oocysts revealed them to be from the genus *Isospora* (K. Townsend, pers. comm.).

2.3.3 Leukocyte counts

A drop of blood was obtained from the right wing of each adult robin via brachial venipuncture to measure leukocyte parameters. Blood was smeared onto a glass slide, fixed

in methanol and stained using a modified May-Grünwald Giemsa staining method (Lucas & Jamroz 1961). Blood smears were then viewed under a light microscope and the following measurements were taken: (1) estimated total leukocyte number (henceforth referred to as leukocyte count) and (2) leukocyte differential. The leukocyte count was calculated by counting all white blood cells in ten consecutive 400x fields of view for each bird. Counts were averaged to give an estimate for each individual (Fudge 2000; Walberg 2001). A leukocyte count gives an indication of the health status of the individual at the time of sampling. A high count, or leukocytosis, is characteristic of inflammatory diseases and parasitic infection (Woerpel & Roskopf 1984; Fudge 2000). Although avian blood is comprised of five types of leukocyte, I focused on the two most common: lymphocytes and heterophils. Lymphocytes are divided into two cell types; T lymphocytes, which play a role in the cell-mediated immune response, and B lymphocytes which are involved in the humoral immune response and antibody production. Both cell types work to generate a pathogen-specific immune response; however, due to the difficulty in distinguishing the two cell types in peripheral blood smears, for the purpose of this study I have treated them as one cell type. A high lymphocyte count is correlated with marked immune stimulation (Fudge 2000); however, a low lymphocyte count can indicate either immunosuppression, viral infection, severe stressors or a lack of parasitic infection (Ots & Horak 1998; Horak *et al.* 1999; Fudge 2000). Heterophils are phagocytic cells and high numbers can indicate either inflammation or stress (Fudge 2000). They play a key role in initiating the innate immune response and the recognition of pathogens by detecting molecules unique to invading organisms (Swaggerty *et al.* 2005). To obtain a differential leukocyte count and thus estimate both lymphocyte and heterophil numbers, each blood smear was examined

under oil immersion (1000x) and the relative frequency of the five different types of leukocytes determined for a total of 100 leukocytes. I then calculated the heterophil/lymphocyte ratio (H/L) which has been used as an index of stress in both poultry (Gross & Siegel 1983; Maxwell 1993) and wild birds (Tompkins *et al.* 2006). Finally, the blood smear was scanned for three minutes to detect any blood borne parasites. A cross-sectional (up-across-down-across-up, etc.) method of scanning the slide was used to prevent scanning the same area twice.

2.3.4 Cell-mediated Immune assay

The phytohaemagglutinin (PHA) skin test is a standard procedure for quantifying one aspect of avian acquired immunity, the T-cell mediated immune response, to a novel challenge. The cell mediated immune system is largely responsible for removing virus-infected host cells and is involved in the defence against fungi, protozoans, cancers, and intracellular bacteria (Ritchie *et al.* 1994). The PHA test works by activating white blood cells in the peripheral blood and causes temporary inflammation at the point of injection. The resultant swelling can then be measured. A large swelling is considered a strong immune response and may provide a measure of the health and condition of the birds (Norris & Evans 2000).

I followed the protocol of Smits *et al.* (1999) by injecting 50uL of a 5mg ml⁻¹ PHA suspension [Sigma (St. Louis, Missouri) PHA-P; L8754] in phosphate buffered saline (Sigma P4244) into the left patagium of adult robins. All robins were held in cages for the six hours between injection of PHA and the final measurements. Cages measured 300 mm by 600 mm and birds were provided with *ad libitum* water, mealworms and a perch. Cages

were placed in a quiet and shady location for the duration of the experiment. I measured a total of 45 adult robins on Nukuwaiata and 101 adult robins on Motuara. Robins were tested in both the spring and autumn to determine any seasonal affect on immunocompetence. Birds were banded to ensure that no individual was tested twice.

I measured patagium thickness at the site of injection to the nearest 0.01mm using a digital micrometer (Mitutoyo 0-1 inch, Tokyo, Japan). Three measurements were taken immediately prior to the injection and again six hours post injection. To reduce measurement error, all measurements were conducted by myself. The three measurements were averaged because wing web thickness is shown to have high repeatability (Moreno *et al.* 1999). The cell-mediated immune response was calculated as the difference between the pre- and post-injection measurements of patagium thickness. For some individuals the difference between pre- and post-injection patagium thickness was negative. The reason for this is unknown, but is likely due to measurement error or because these individuals became dehydrated during the post-injection holding period (J. E. Smits, pers. comm.). Although all individuals were supplied with water I was not able to control the amount they consumed. Statistical analyses were run both with and without these individuals included, but it had no effect on the outcome of the analysis and I only present the results with all individuals included.

2.3.5 Statistical analyses

I performed all statistical analyses in Statistica 6 (StatsSoft, Inc.). I used general linear models to determine which variables explained a significant proportion of variation in the cell mediated immune response, the leukocyte counts, the counts of lymphocytes and

heterophils, and in the H/L ratio. I used the non-parametric Kruskal-Wallis test to determine seasonal variation in parasite abundance across both islands and also within island by season comparisons. Pearson correlations were used to determine whether the H/L ratio correlated with leukocyte count or PHA response for island by season combinations.

2.4 Results

2.4.1 Parasite loads and bottleneck size

Robins in both populations were found to harbour feather mites and hippoboscids flies, but there was no significant difference in the prevalence of ectoparasites between the source population on Nukuwaiata and the bottlenecked population on Motuara Island in either the autumn or spring (Fig. 2.1). Hippoboscids numbers were significantly higher in the autumn than in the spring ($H_{1, 146} = 73.43$, $P < 0.001$), and this pattern held for both islands when analysed separately (Motuara: $H_{1, 101} = 59.33$, $P < 0.001$; Nukuwaiata: $H_{1, 45} = 14.80$, $P = 0.001$). In contrast, feather mite loads were higher in the spring than autumn ($H_{1, 143} = 90.82$, $P < 0.001$) and this was true for both islands (Motuara: $H_{1, 98} = 67.30$, $P < 0.001$; Nukuwaiata: $H_{1, 45} = 26.17$, $P < 0.001$). Ectoparasite loads were also not correlated with either leukocyte counts or PHA responses (Pearson correlation coefficients, all $P > 0.05$). Coccidia was the only endoparasite isolated from faecal samples, but it was only present in 3/101 (3.0%) individuals on Motuara Island and 1/45 (2.2%) individuals on Nukuwaiata Island. Oocyst counts of coccidia were low to moderate (200-2600 oocysts/gram) in all infected individuals. No nematodes were found and no blood parasites were seen from the blood smears in any robin from either population. Thus, there was no indication from surveys of parasites that robins in the severely bottlenecked population presently suffer significantly greater loads of parasites than do birds in the source population.

2.4.2 Blood cell counts and bottleneck size

Blood parameters differed in a number of ways between the bottlenecked population on Motuara Island and their source population on Nukuwaiata Island (Fig. 2.2). Leukocyte

counts were significantly different between seasons ($F_{1,130} = 76.81$, $P < 0.001$) and sexes ($F_{1,130} = 8.31$, $P < 0.05$), but robins on Nukuwaiata Island had a significantly higher leukocyte count than robins on Motuara Island ($F_{1,130} = 13.28$, $P < 0.001$; Fig. 2.2). Similarly, there was a significant difference in lymphocyte count between seasons ($F_{1,130} = 95.65$, $P < 0.001$) and sexes ($F_{1,130} = 7.77$, $P < 0.01$), and robins on Nukuwaiata Island again showed significantly higher counts than birds on Motuara Island ($F_{1,130} = 12.82$, $P < 0.001$; Fig. 2.2). In contrast, heterophil counts did not differ significantly between the two populations or sexes, although they were higher in autumn than spring for both islands (Fig. 2.2). There was also no difference in H/L ratio for either island or season (Fig. 2.2). Within each island and season, H/L ratios were not correlated with leukocyte counts (Pearson correlation coefficients, all $P > 0.05$) indicating that the leukocyte response was not due to one particular type of leukocyte. PHA response was also not correlated with H/L ratio, leukocyte count or heterophil or lymphocyte counts within each island or season (Pearson correlation coefficients, all $P > 0.05$). The higher leukocyte and lymphocyte counts in the source population suggests that robins in the bottlenecked population have a reduced immune response although this reduction was not evident in other measures such as heterophil counts or H/L ratios.

2.4.3 Cell mediated immune response and bottleneck size

Although there was no overall difference between the cell mediated immune response in the PHA experiment between Motuara and Nukuwaiata islands ($F_{1,138} = 1.20$, $P = 0.28$), there was a significant interaction between population and season ($F_{1,138} = 11.90$, $P < 0.001$). When each season was examined separately, robins in the source population on

Nukuwaiata Island had a significantly higher cell mediated immune response in the autumn than robins in the bottlenecked population on Motuara Island ($F_{1,81} = 10.47$, $P < 0.05$; Fig. 2.3). However, the response of birds did not differ significantly between the two populations in the spring ($F_{1,57} = 2.80$, $P > 0.05$; Fig. 2.3). These results confirm the cell-mediated immune system of robins in the bottlenecked population was weaker than in the source population when experimentally challenged but that this difference varied with the season.

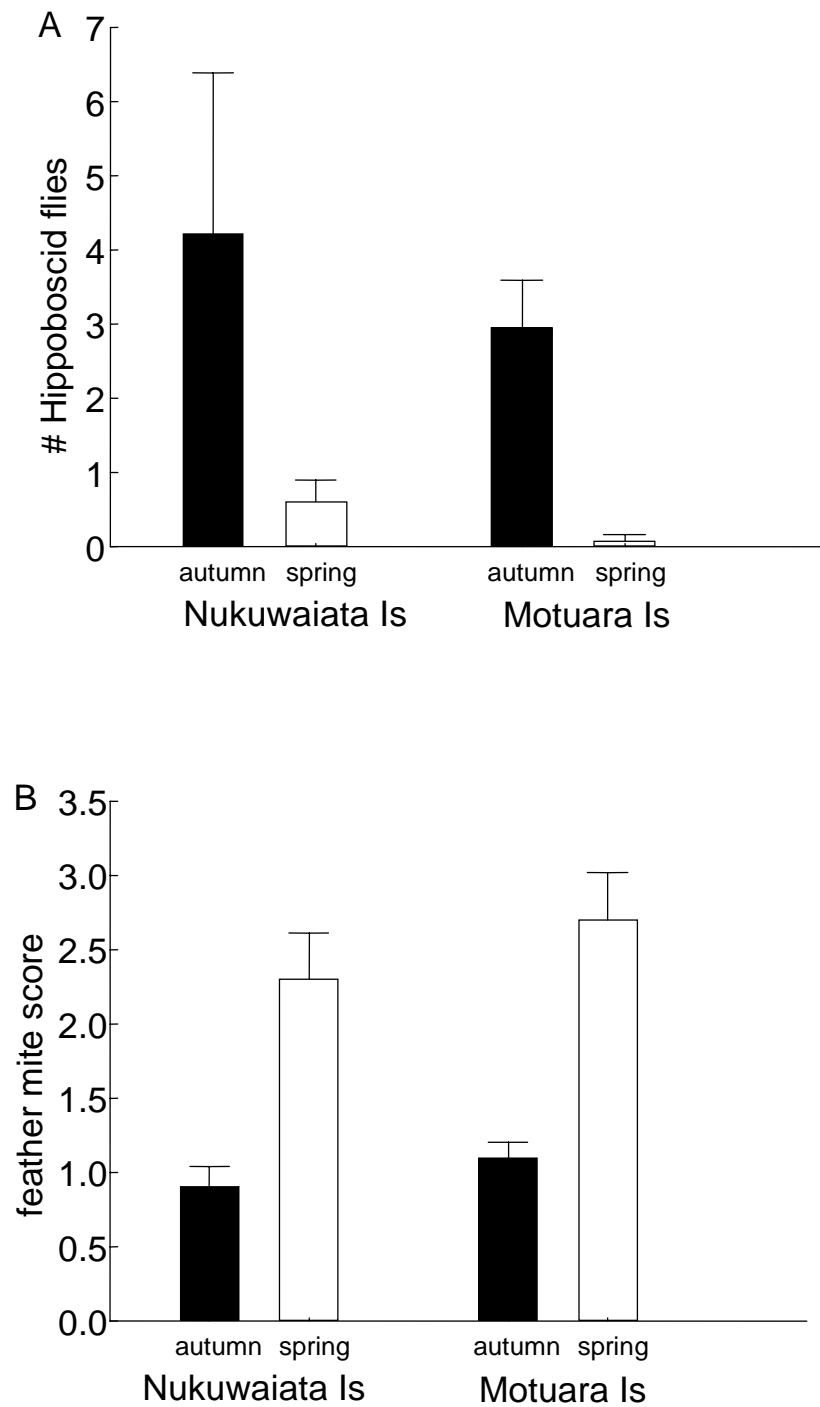


Figure 2.1 Difference in parasite loads between autumn and spring for New Zealand robins in source population on Nukuwaiata Island compared to birds in bottlenecked population on Motuara Island.

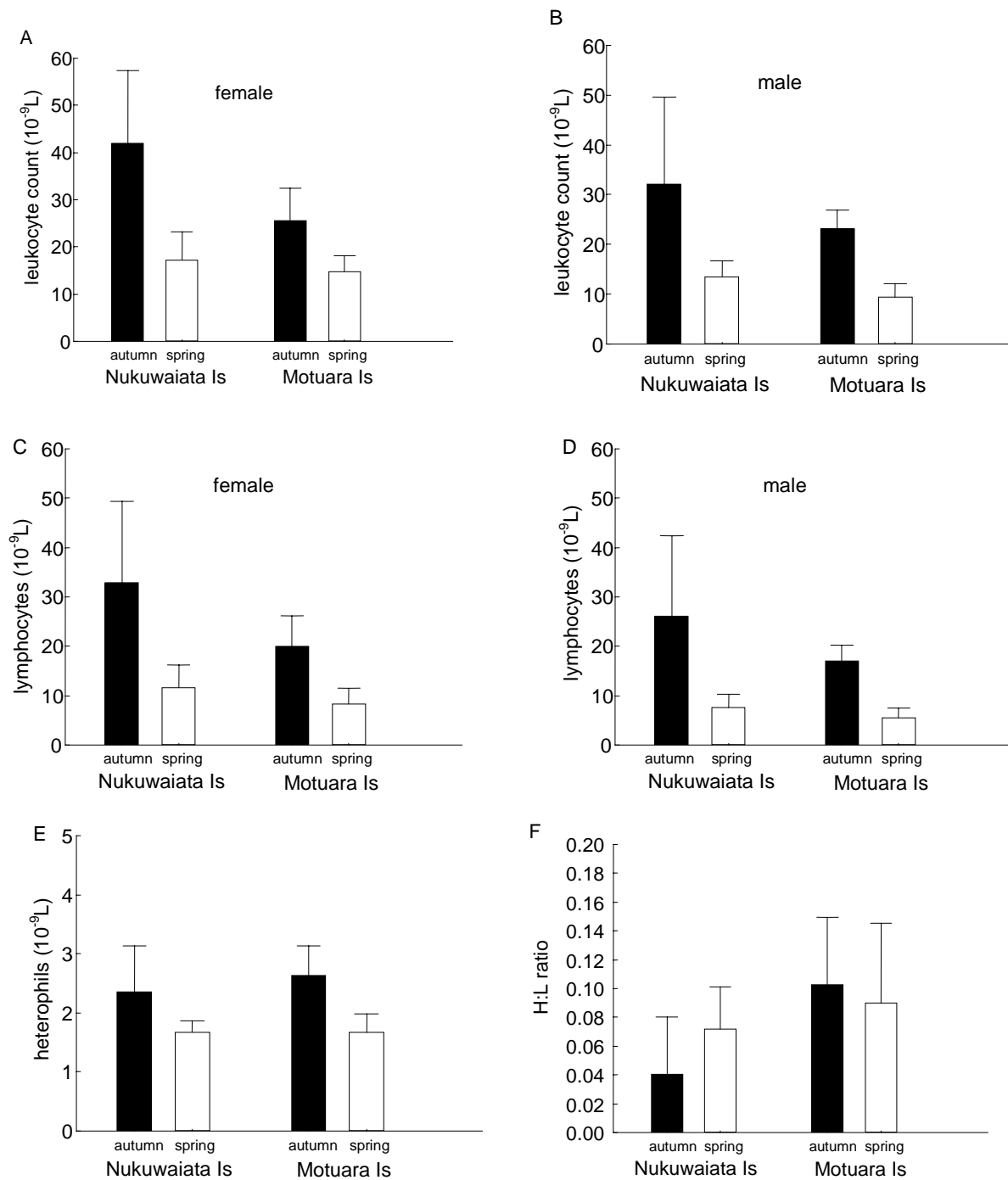


Figure 2.2 Blood profiles showing relationships between leukocyte counts for females (A) and males (B) in the autumn (closed bars) and spring (open bars) for New Zealand robins in source population on Nukuwaiata Island and their bottlenecked population on Motuara Island; Lymphocyte counts for females (C) and males (D) during both seasons and on both islands; (E) heterophil count and (F) H:L ratio in females and males during both seasons and on both islands.

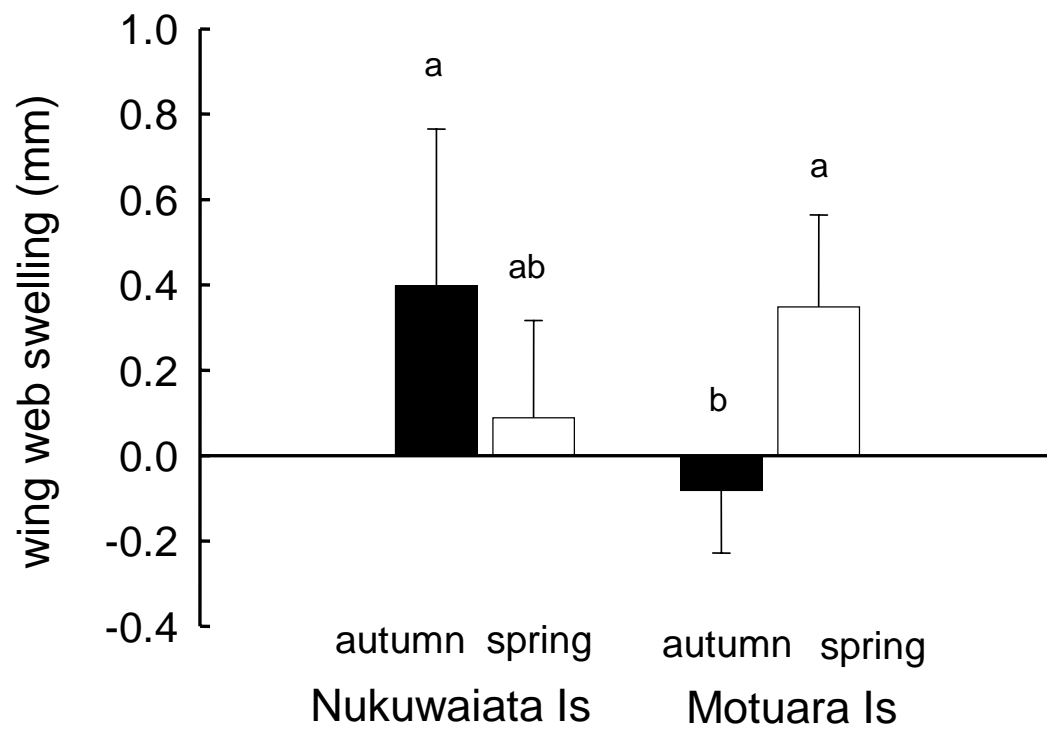


Figure 2.3 PHA response, calculated as difference between pre- and post-PHA injection, for New Zealand robins in source population on Nukuwaiata Island compared to robins in bottlenecked population on Motuara Island during autumn (black bars) versus spring (open bars). The same letters above bars indicate no significant difference between bars.

2.5 Discussion

The genetic consequences of severe population bottlenecks are well documented (e.g. O'Brien *et al.* 1985; Saccheri *et al.* 1998; Westemeier *et al.* 1998; Briskie & Mackintosh 2004). Even if such populations subsequently recover through conservation initiatives, individuals in the post-bottlenecked population are expected to have reduced genetic diversity through inbreeding and genetic drift (Frankham *et al.* 2002; Reed & Frankham 2003). Whether such a loss is manifested in decreased fitness has been less clear but increased inbreeding as a result of a bottleneck should lead to increased susceptibility to pathogens and a compromised immune system (Keller *et al.* 1994; Spielman *et al.* 2004). Several disease outbreaks in endangered wild populations support the link between reduced genetic variation and susceptibility to pathogens (Altizer *et al.* 2001; Spielman *et al.* 2004). For example, feline infectious peritonitis virus in cheetah *Acinonyx jubatus* (O'Brien *et al.* 1985), canine distemper in the black footed ferret *Mustela nigripes* (Thorne & Williams 1988) and a number of infectious diseases found in the Florida panther *Puma concolor coryi* (Roelke *et al.* 1993) have all been linked to the negative effects of bottlenecks experienced by these species.

When I examined a variety of internal and external parasites in a highly bottlenecked population of the New Zealand robin, I found no differences in parasite loads compared with that found in their source population in either the pre-breeding or post-breeding seasons. However, at a cellular level, the immune systems of robins appeared to be less responsive in the severely bottlenecked population. Robins in the bottlenecked population on Motuara Island had significantly fewer leukocytes (including fewer lymphocytes) than their counterparts in the source population on Nukuwaiata Island, and

when the immune systems of robins were experimentally challenged with PHA, I again found that birds in the severely bottlenecked population responded less strongly than birds in the source population, at least in one season. My experimental results thus add to the growing body of evidence (e.g. Reid *et al.* 2003; Hawley *et al.* 2005; Whitman *et al.* 2006) that populations passing through a recent severe bottleneck have a lower cell-mediated immunocompetence and may therefore be more susceptible to parasites and diseases.

The lack of a difference in current parasite loads on birds in both populations would at first glance run counter to my expectations based on reduced genetic diversity in a bottlenecked population: if severe bottlenecks reduce immunocompetence one would expect birds in the post-bottlenecked population to be particularly prone to infestation and therefore carry higher burdens. However, using current pathogen loads to infer the strength of a bird's immune system is subject to errors that can mask any underlying difference in immunocompetence. For example, severe infestations might quickly lead to mortality and such birds would be under-represented in any survey of either population, either through death or a reduced likelihood of capture. Likewise, some pathogens are only likely to become costly when an individual is stressed, and as I only sampled birds in the non-breeding season (when birds are probably under less stress than in the breeding season), it is possible that pathogen loads differ at other times of the year. Finally, for practical reasons I could not assess every possible pathogen or parasite (e.g. tapeworms, flukes) and it is possible that other types of parasites and pathogens might differ between the two populations.

The fact that I found similar parasite loads and H/L ratios in both the source and bottlenecked robin populations suggests that neither population is currently subject to

greater environmental stresses than the other. Such a difference might be expected when comparing two populations as defence against infections, including acquired immunity, can be modulated by environmental factors such as nutrition, stress and age (Gustafsson *et al.* 1994) and these are likely to be different in different study sites. Furthermore, extreme environmental conditions are known to increase susceptibility to disease and parasites (Hoffmann & Parsons 1991). Thus, differences in levels of environmental stress could be a confounding factor in many comparative studies of immunocompetence. However, the close proximity of my two populations means they share similar environmental conditions, temperatures, and habitat structure. Both are also located on small offshore islands with similar size and geography. This suggests that the differences I observed in cell-mediated immunocompetence are likely the result of a severe population bottleneck, rather than different levels of stress that might be associated with differing environmental conditions between the two study sites.

As elevated leukocyte counts are indicative of an individual mounting an immune response (Fudge 2000), the higher leukocyte counts of robins in the source population would suggest that either they are responding more effectively to immune challenges than birds in the post-bottlenecked population or that birds in the latter population are simply unable to respond as effectively. Alternatively, the difference in leukocyte counts could arise from one population dealing with a disease epidemic not found in the other population. However, I found no physical evidence, as would be expected with such an epidemic, that either population was currently suffering from a disease outbreak of any sort. The results of the PHA test, in which I experimentally challenged the cell-mediated immune system of birds in both populations, suggests that the lower leukocyte counts in the

post-bottlenecked population are more likely due to the inability of robins to mount as strong an immune response as their counterparts in the source population, even when faced with apparently similar parasite loads. Further information on how leukocyte counts change in response to specific pathogens (e.g. coccidia) is now needed to determine whether reductions in immunocompetence occur uniformly across different types of immune system challenge (Matson *et al.* 2006).

One striking result of my study was the seasonal changes in the reaction of robins to the PHA immune challenge. Robins in the post-bottlenecked population displayed a reduced immune response only in the autumn and not in the early spring before breeding began. Seasonal differences in parasite loads were also noted, with hippoboscids more common in the autumn and feather mites more common in the spring. Such seasonal differences in immunocompetence and parasite loads have been noted previously, and it has been suggested that this pattern results from a trade-off between increased reproductive effort and reduced immunocompetence (Deerenberg *et al.* 1997; Merino *et al.* 2000; Dubiec & Cichon 2001; Lozano & Lank 2003; Møller *et al.* 2003). However, Møller *et al.* (2003) suggest that seasonal differences in immune response reflect the impact of parasites on their hosts and that it would be beneficial to mount an immune response when parasites are most abundant. The numbers of some parasites tend to peak during and just after the reproductive season of their host (Møller *et al.* 2003). The high numbers of hippoboscids I found on robins fits with this pattern and may explain the higher response to the PHA at this time of the year. However, further information on the immune response of birds across the entire year and in relation to their condition is needed before the significance of seasonal fluctuations is better understood. My results nevertheless provide a caution to

other researchers drawing conclusions on the immunocompetence of birds based on data from only one season.

The immune system of birds is complex and there is no single assay available that can assess all kinds of immune response simultaneously. Because I only challenged one aspect of the acquired immune system I cannot rule out the alternative that resources were directed towards different immune components in the Motuara robin population. For example, more resources may have been invested in the humoral immune response than the T-cell mediated immune response during the autumn period (Westneat & Birkhead 1998; Norris & Evans 2000; Adamo 2004) and this could explain the difference between my two study populations. However, the low reaction to PHA and low leukocyte counts suggest that the robins from the post-bottlenecked population on Motuara may be less capable of mounting an immune response in any form. At present, the population on Motuara appears stable and self-sustaining but my results suggest that if a new pathogen appeared on the island it might have more severe consequences than in the source population.

Theoretical evidence suggests that the individuals most likely to survive adverse conditions and disease outbreaks are those with the greatest genetic diversity (Frankham 1995, 1998). Genetic diversity of the immune system is known to cause differences in resistance to infectious pathogens in chickens (Zekarias *et al.* 2002), and two studies on wild animals found that reduced genetic diversity strongly influenced response to PHA (Reid *et al.* 2003; Hawley *et al.* 2005). However, Hawley *et al.* (2005) did not find a relationship between heterozygosity and humoral immune response, suggesting that there may be differences in the way each component of the adaptive immune response is influenced by genetic diversity. In vertebrates, mediator proteins such as the major

histocompatibility complex (MHC) are probably responsible for linking the different events of the immune response and they play a major role in the recognition of viruses (Benjamini *et al.* 2000). The MHC is the most highly polymorphic gene in the vertebrate body (O'Brien *et al.* 1985), and maintaining a large number of MHC genes is thought to increase pathogen resistance. A recent study of MHC variation in the two robin populations used in the present study confirms a loss of MHC diversity in the Motuara Island robins compared to their source population (Miller & Lambert 2004). As disease is known to have been the main contributing factor of population decline and/or extinctions for several endangered species (van Riper *et al.* 1986; Wikelski *et al.* 2004), the loss of MHC diversity may be the genetic mechanism by which bottlenecked populations become more susceptible to pathogens.

The population of robins I studied on Motuara Island went through an extremely severe bottleneck of only five individuals and thus it is not too surprising that their immune systems might be compromised as a result. Such severe bottlenecks have occurred in a variety of endangered species around the world (e.g. black robin *P. traversi*; Butler & Merton 1992; Mauritius kestrel *Falco punctatus*; Groombridge *et al.* 2001) and thus it is important to understand the fitness consequences on individuals in the post-bottlenecked population for effective management. Nevertheless, conservation management of endangered birds usually involves founding new populations with a greater number of individuals (usually 30-75 founders; Griffith *et al.* 1989) than that experienced by New Zealand robins on Motuara Island. In a recent study, Briskie and Mackintosh (2004) found that bottlenecks below ~150 individuals resulted in increased hatching failure, but whether bottlenecks in this higher range also reduces the immunocompetence (and hence survival)

of birds in the post-bottlenecked populations is not known at present. Such information is urgently needed if conservationists are not to unwittingly increase the susceptibility of endangered species to parasites and pathogens as a consequence of their actions of founding new populations without considering the genetic consequences on immunocompetence.

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Chapter 3

The effect of increasing population density on reproductive success and parasite loads in an island population of South Island saddleback (*Philesturnus c. carunculatus*)

3.1 Abstract

Many of New Zealand's endangered bird species survive only on offshore islands free of exotic predators. The small size of most island populations of endangered birds, together with their high population density, low genetic diversity and naivety to introduced parasites and pathogens, may render many island populations vulnerable to disease outbreaks and extinction. Such an outbreak occurred in an isolated population of South Island saddleback (*Philesturnus c. carunculatus*) on Motuara Island, in the Marlborough Sounds, New Zealand. In early 2002, the population dropped from approximately 140 birds to less than 50 birds, apparently from an outbreak of systemic coccidiosis, a disease caused by the protozoal parasite coccidia. However, over the next three years the population rebounded to > 150 individuals. This gave me the opportunity to monitor any changes in reproductive success and parasite loads as the population density increased three-fold to the apparent carrying capacity of the island. The reproductive success of saddlebacks was similar for all three years following the crash and there were no differences in clutch size, nesting success or fledgling weight as the population rapidly increased to its pre-crash density. Nevertheless, by the time the population recovered, adult birds had significantly higher feather mite loads, H/L ratios and heterophil counts, and significantly lower lymphocyte counts compared to birds immediately after the population crash when density was much lower. These findings suggest that although reproductive success does not appear to be affected by the changes in population density in the ranges I studied, there may be an increasing risk of parasite transmission at high population densities as well as increasing levels of stress and immunosuppression in individuals, and this could render small island populations more susceptible to disease outbreaks.

3.2 Introduction

Disease has been a neglected topic in conservation and it is only recently that exotic diseases and parasites have been highlighted as important driving factors of population declines and extinctions in many plants and animals (Friend *et al.* 2001; McCallum & Dobson 1995; Spielman *et al.* 2004). One of the most well documented examples of the impact of disease on population dynamics is the extinction of over half the Hawaiian terrestrial bird species due to the introduction of avian malaria and avian pox (Warner 1968; van Riper *et al.* 1986). Parasitic organisms are now considered one of the greatest threats to the survival of many endangered species (Altizer *et al.* 2001).

Inbreeding depression has been recognised as an important factor contributing to increased disease susceptibility in a number of wild and captive populations. For example, feline infectious peritonitis virus is more common in inbred captive cheetah (*Acinonyx jubatus*; O'Brien *et al.* 1985), and canine parvovirus (CPV-2) is more common in grey wolves (*Canis lupus*) in the isolated population on Isle Royale (Peterson *et al.* 1998). There is growing evidence to suggest that inbred populations exhibit lower levels of immunocompetence than those with comparatively higher levels of heterozygosity (Reid *et al.* 2003; Hawley *et al.* 2005; Hale & Briskie 2006). The loss of genetic variation means populations are less adaptable to environmental change (Frankham 1996; Meagher *et al.* 2000; Keller & Waller 2002; Hedrick & Kalinowski 2003), thus the combination of inbreeding and environmental stress may prove to be highly detrimental to many endangered populations and render them more susceptible to disease outbreaks.

It has long been suspected in zoos that inbreeding, crowding and stress are three important factors in increased disease susceptibility of individuals (Altizer *et al.* 2001; Keller &

Waller 2002). Yet such factors are only now being considered as main contributors to the increase in disease susceptibility amongst wild populations.

Island populations in particular are expected to be more prone to disease outbreaks and extinction than mainland populations largely owing to inherent low genetic diversity due to bottlenecks during their establishment and because simple ecosystems such as islands are more susceptible to invasion by new diseases (Frankham 1998; Altizer *et al.* 2001). Indeed, many examples of the catastrophic effects of introduced parasites in small populations come from island populations (Altizer *et al.* 2001). However, island populations tend also to exist at high density as a result of the small size and isolation of many island habitats. High densities may exacerbate the effects of inbreeding, elevating stress levels and further reducing an already low threshold above which a population may succumb to disease and adverse environmental conditions; the result can be population crashes and possible local extinction (Miller 1994; Wikelski *et al.* 2004).

A recent crash of a South Island saddleback (*Philesturnus carunculatus* *carunculatus*) may provide one such example of the vulnerability of isolated island populations to pathogens. In March 2002 a survey of the population revealed it had declined from over 100 individuals to less than 50 within a period of 1-2 months (pers. obs.). Individuals captured at this time were found to be infested with hippoboscids flies and some exhibited lesions on the face, cloaca and wings. Autopsies from deceased saddlebacks revealed the birds were infected with severe systemic coccidiosis. Prior to the crash the population was believed to be close to the carrying capacity of the island (approx. 130 individuals: Fig. 3.1) (Hooson & Jamieson 2003). This population therefore provides

an opportunity to investigate the potential role high population density may have had in the sudden decline of this saddleback population.

By monitoring the Motuara Island saddleback population for three years immediately following the crash my main aims are to determine: 1) whether the population recovers, 2) whether increased density over time affects various measures of reproductive success, 3) whether increasing density leads to increased parasite burdens and, 4) whether increasing density affects the immune response of birds as assessed by leukocyte counts.

3.3 Methods

3.3.1 Study population

The South Island saddleback is a medium-sized, forest passerine of the endemic wattlebird family Callaeidae, which also includes the North Island saddleback (*P. c. rufusator*), kokako (*Callaeas cinerea*) and the extinct huia (*Heteralocha acutirostris*). Both male and female saddlebacks have jet-black plumage with a highly conspicuous chestnut-coloured saddle on the back and fleshy orange wattles on either side of the bill. Juvenile South Island saddlebacks lack this distinct colouration and are instead a rusty brown colour until they moult into adult plumage at two years of age, making them readily distinguishable from adults. Saddlebacks are poor fliers, and feed mainly on the forest floor on a diet of invertebrates and fruits (Heather & Robertson 2000).

Saddlebacks were once widespread across New Zealand however both sub-species rapidly declined with the introduction of mammalian predators, particularly rats, in the nineteenth century. By 1964 only 36 South Island saddlebacks remained on a single island, Big South Cape, a remote island situated south west of Stewart Island. Numerous translocations have now created 15 additional island populations of this sub-species and all were established from this original population or its descendants. For example, in March 1994, 26 saddlebacks were translocated from Jacky Lee and North islands (both in the area around Stewart Island) and released on the Motuara Island (59 ha) in the Marlborough Sounds of the South Island, the northern most limit for this subspecies. This island was selected for the release of South Island saddlebacks because this species was believed to be historically resident in the area and the island was free of introduced predators after the eradication of Polynesian rats (*Rattus exulans*) in 1993. By 2001, the population had

reached over 130 birds and was nearing the carrying capacity of the island (Hooson & Jamieson 2003).

3.3.2 Population size

Population census data for the years 1994 to 2001 was collated from published data (Hooson & Jamieson 2003; Lovegrove 1996) and from personal communication with the Department of Conservation (W. Cash, Marlborough Conservancy). Population surveys for the periods 2002 to 2006 were obtained by myself and other researchers by carrying out extensive searches of 90% of the island over a period of six months of each year. Surveys began at the start of the breeding season and continued until all known nestlings had fledged. The final population estimate recorded for each year was taken at the beginning of autumn (April/May) when the breeding season was complete. All population surveys included counts of adults and juveniles. Most saddlebacks were colour-banded for individual identification but because a small percentage (approximately 10-20% per year) of individuals remained unbanded, it is possible some individuals may have been counted twice. However, saddlebacks from the age of 2 years are typically very territorial and young birds from that season stay within the parent's territory until the winter when they disperse. Over the breeding seasons between 2002 and 2006 I was able to identify these territories and observe the behaviour and movement of individuals on the islands, making it less likely that an unbanded individual was counted more than once.

3.3.3 Reproductive success

I searched all accessible areas on Motuara Island (approximately 90% of the island) for the three breeding seasons from October 2002- April 2005 to locate South Island saddleback nests. This period follows the three years immediately after the population crash in March 2002. Nests were flagged with numbered tape and visited every two to three days to monitor their progress. Nestlings were weighed every two days from hatching, or from the time the nest was found if eggs had already hatched, to the stage when nestlings were fully feathered (approximately day 17). On the last visit each nestling was banded with a colour combination to code for the hatch year of the individual (for example, all nestlings from 2002 were banded white over metal). A final weight was obtained to determine the pre-fledge mass of the individual and a blood sample was taken for analysis of blood parameters (see below). A post-fledge weight was obtained from fledglings caught at the end of breeding season between the months February-April each year. At this stage, sexes are distinguishable by differences in tarsus length and body mass (Nillson 1978). Thus, post-fledgling body mass was analysed separately for males and females.

Egg success, nest success and annual reproductive success were calculated following the protocol outlined by Bertram (2000). Egg success, the proportion of eggs that produce young, was calculated as the number of young that leave the nests divided by the total number of eggs laid. Nest success, the proportion of clutches that produce young, was calculated as the total number of clutches that produce young divided by the total number of clutches. Annual reproductive success (ARS) was calculated as the number of broods successfully reared per female (defined as ARS [b]) and the number of young successfully reared per female (defined as ARS [k]; see (Bertram 2000) for details).

3.3.4 Parasite analysis

Between November 2002 and August 2005, I caught 108 adult and sub-adult (>1 year but <2 years of age) saddlebacks for the analysis of parasite loads. I caught birds with mist nets and all birds were banded with a metal band in accordance to the New Zealand national banding scheme. For the purposes of analysis, I have broadly defined two sampling seasons: spring/summer (October – March) and autumn/winter (April – September). This corresponds with the breeding and post-breeding season, respectively. An estimate of feather mite density was obtained for each individual by examining the primary feathers of the left wing and counting the number of mites visible to the naked eye. I estimated feather mite density by giving a categorical score for each bird from 0 – 5, where: 0 = no feather-mites; 1 = 0-10 feather mites; 2 = 10-100; 3 = 100-1000; 4 = 1000-10000 and 5 = 10000+ feather mites. The number of hippoboscids flies (*Ornithomya* spp and *Ornithoica* spp) seen on or flying off the bird were also counted. Hippoboscids flies are obligate blood-feeding ectoparasites and are known to be vectors of a number of blood borne diseases (Hutson 1984). Faecal samples were collected from holding bags (the birds usually defecated while being held in cloth bags) for analysis for gastrointestinal nematodes and the protozoan parasite coccidia. Faecal floatation was used to obtain estimates of coccidia and nematode burdens and these were carried out at a commercial laboratory (New Zealand Veterinary Pathology Ltd, Hamilton). Sporulation of the coccidia oocysts was additionally carried out in order to identify the genus of the species.

3.3.5 White blood cell analysis

To measure leukocyte parameters, I collected a drop of blood from the right wing of each individual via venipuncture of the brachial vein. Blood was smeared onto a glass slide, fixed in methanol and stained using a modified May-Grünwald Giemsa staining method (Lucas & Jamroz 1961). Blood smears were then viewed under a light microscope and the following measurements taken: (1) estimated total leukocyte number (leukocyte count), and (2) leukocyte differential. The leukocyte count was calculated by counting all white blood cells in ten consecutive fields of view for each slide at a magnification of 400 X. Counts were averaged to give an estimate for each individual (Walberg 2001). To obtain a differential leukocyte count and thus estimate numbers of lymphocytes, heterophils, basophils and eosinophils, each blood smear was examined under oil immersion (1000 X magnification) and the relative frequency of the five different types of leukocytes determined for a total of 100 leukocytes. I surveyed the blood smear using a cross-sectional method (down-across-up-across-down etc.) identifying each type of leukocyte when encountered until a total of 100 leukocytes were counted. The heterophil/lymphocyte ratio (H/L), which has been used as an index of physiological stress in both poultry (Gross & Siegel 1983; Maxwell 1993) and wild birds (Tompkins *et al.* 2006), was then calculated by dividing the number of heterophils by the number of lymphocytes. Finally, I scanned the blood smear for three minutes using the same cross sectional method to detect any blood borne parasites.

3.3.6 Statistical analysis

I performed all statistical analyses in Statistica 6 (StatsSoft, Inc.). I used general linear models to determine if there was significant variation in leukocyte counts, the counts of lymphocytes, heterophils, eosinophils and basophils between age groups, years, seasons and sexes. I used the non-parametric Kruskal-Wallis test to determine any differences in the various measures of reproductive success, H/L ratio and parasite counts between years. I used one-way ANOVAs to determine if pre- and post-fledgling juvenile weight differed between years. A Fisher exact test was used to determine if there was any significant difference in survival of fledglings from the first two breeding seasons (2001/02 and 2002/03).

3.4 Results

3.4.1 Population recovery after the crash

The population surveys following the crash showed a rapid recovery of the Motuara Island saddleback population with the number increasing from 50 to 104 individuals in 2003, and to 119 individuals in 2004. By 2005, the final year of my surveys and 3 years after the crash, the population was estimated to be at 158 individuals, exceeding the pre-crash total of 130 individuals (Fig. 3.1).

3.4.2 Changes in reproductive success between years

Overall reproductive success did not differ among the three years following the population crash, however second broods were only found for the 2003/04 and 2004/05 breeding seasons (Table 3.1). Mean clutch size, the number of fledglings per successful nest, egg success, and nest success were not significantly different across the three breeding seasons (Kruskal-Wallis test, all $P > 0.05$: Table 3.1). Similarly, annual reproductive success in terms of females as estimated by ARS(b) and ARS(k), did not differ significantly across the three breeding seasons (Kruskal-Wallis test, both $P > 0.05$: Table 3.1). There was also no significant difference between breeding seasons for juvenile pre-fledge ($F_{2,61} = 0.10$, $P > 0.05$) or post-fledge (males: $F_{2,13} = 1.70$, $P > 0.05$; females: $F_{2,16} = 2.95$, $P > 0.05$) weights (Table 3.1). Survival estimates of fledglings from the first breeding season and the second breeding season were 11/35 (51%) and 18/35 (31%) respectively. However, a Fisher exact test revealed no significant difference between these two survival estimates (two-tailed, $P = 0.11$).

3.4.3 Changes in parasite loads between years

Hippoboscids were found on adult saddleback over all three years; however there was no significant difference in prevalence between years ($H_{2,104} = 5.45$, $P > 0.05$) with the number of flies per individual averaging between two and four (Fig. 3.2a). In contrast, there was a significant difference in feather mite scores between years with individuals exhibiting significantly higher loads in the third year following the crash compared to the first ($H_{2,104} = 5.45$, $P < 0.01$; Fig. 3.2b). There was also a significant difference between years in coccidia prevalence ($H_{2,60} = 18.29$, $P < 0.001$) with coccidia oocysts isolated from faecal samples in the year following the crash and none from samples collected in the second and third years (Fig. 3.2c). I detected no blood parasites from the blood smears for any individuals from any population.

3.4.4 Changes in leukocyte counts between years

Nestlings

A change in a number of leukocyte parameters was exhibited when comparing nestlings across the three breeding seasons after the population crash. Nestlings that hatched during the first breeding season following the crash had a significantly lower total leukocyte count ($F_{2,54} = 15.74$, $P < 0.001$) compared to the subsequent two years (Fig. 3.3). The number of lymphocytes ($F_{2,54} = 13.34$, $P < 0.001$), heterophils ($F_{2,54} = 29.12$, $P < 0.001$) and eosinophils ($F_{2,54} = 6.38$, $P < 0.01$) were also significantly lower in nestlings hatched in the year after the crash compared to the following two breeding seasons (Fig. 3.3). In contrast, basophil numbers in nestlings from the second breeding season were significantly higher compared to those from either the first or third breeding seasons ($F_{2,55} = 17.33$, $P < 0.001$). I found no significant difference in H/L ratio between years (Fig. 3.3).

Adults

There was no difference in total leukocyte counts, eosinophil numbers or heterophil numbers between the three years, sexes or seasons (all $P > 0.05$: Fig. 3.4). However, a number of differences were seen in lymphocyte and basophil counts when compared among the three years following the crash. As seasonal differences were also observed in these blood parameters, I analysed each season separately. Lymphocyte counts were significantly different between seasons with lower counts of lymphocytes in autumn/winter compared to spring/summer ($F_{1,82} = 6.74$, $P < 0.05$). When I compared years within each season, individuals from the third year after the crash had significantly lower lymphocyte counts in the autumn/winter compared to individuals within year one and two after the crash ($F_{2,54} = 9.20$, $P < 0.001$: Fig. 3.4). However, there was no significant difference in lymphocyte counts between years in the spring/summer ($F_{2,34} = 0.771$, $P > 0.05$) and there was also no difference between the sexes. Similarly, there was a significant difference between years in counts of basophils, with significantly higher basophil counts in year three compared to years one and two ($F_{2,84} = 3.45$, $P < 0.05$). While there was no overall significant effect of season on basophil counts, there was a significant interaction between year and season ($F_{2,84} = 3.39$, $P < 0.05$). When I examined each season separately I found significantly higher basophils counts for year three compared to year one and two for the spring/summer period ($F_{2,36} = 5.12$, $P < 0.05$: Fig. 3.4), but there was no difference in basophil counts between years for autumn/winter ($F_{2,54} = 0.211$, $P > 0.05$). There was also a significant season and sex interaction with a higher basophil count for males compared to females for spring/summer ($F_{1,84} = 5.13$, $P < 0.05$). Finally, individuals from

the third year were found to have significantly higher H/L ratios in autumn/winter than in the previous two years ($H_{2,56} = 13.22$, $P < 0.01$: Fig. 3.4), however there was no difference between years for spring/summer ($H_{2,38} = 4.00$, $P > 0.05$: Fig. 3.4).

Table 3.1 Measures of reproductive success in the Motuara Island saddleback population

Breeding season	# breeding females	Total # nest attempts monitored	Reproductive success					fledgling body mass (g)			
			Mean clutch size	Egg success	Nest success	# fledglings per nest	ARS(b)	ARS(k)	Pre-fledge (~ 17 days)	Post-fledge male	Post-fledge female
2002	16	19	1.89	0.75	0.76	1.86	0.906	1.69	62.65	79.67	73.22
2003		23	1.86	0.77	0.76	1.89					
		4	1.85	0.71	0.75	1.67					
	21	27	1.85	0.76	0.76	1.85	0.976	1.81	62.93	84.00	74.60
2004		19	2.00	0.76	0.79	1.93					
		3	1.67	0.80	0.83	1.6					
	18	22	1.95	0.77	0.80	1.89	0.972	1.78	63.25	80.90	70.29

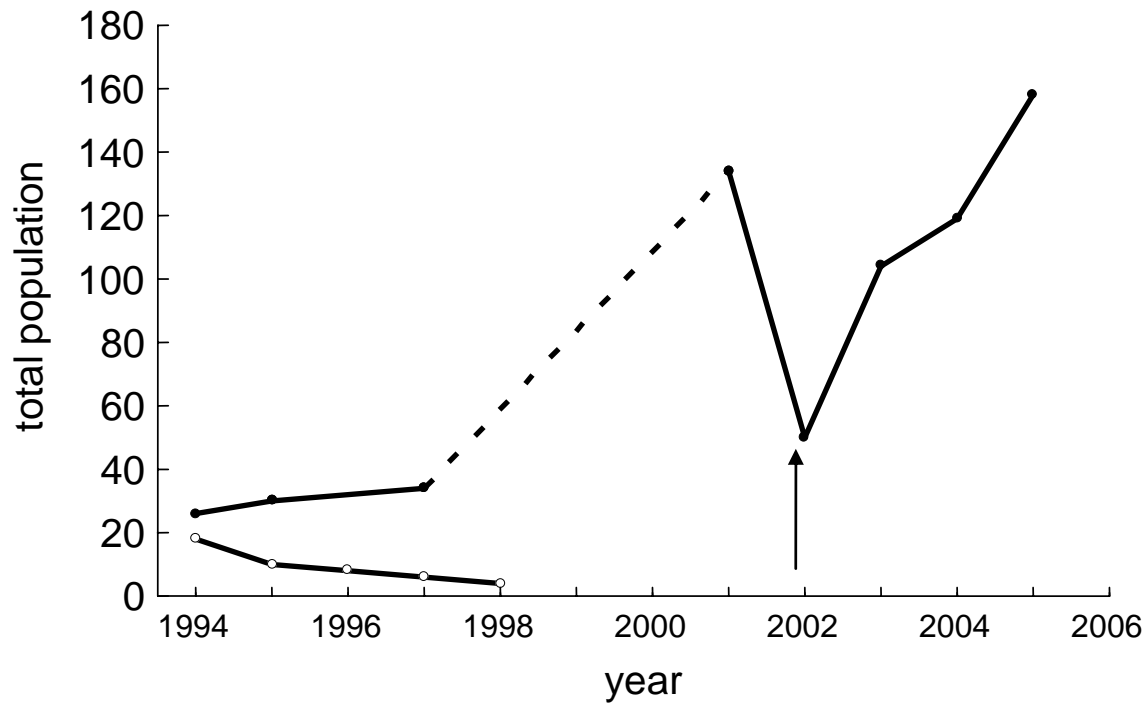


Figure 3.1 Population growth of the Motuara Island saddleback population from the year of reintroduction to present (closed circle solid line) and decrease in original founders (open circle solid line). The arrow indicates the period in which the population crash occurred. The dotted line represents the period over which no population density estimates were available.

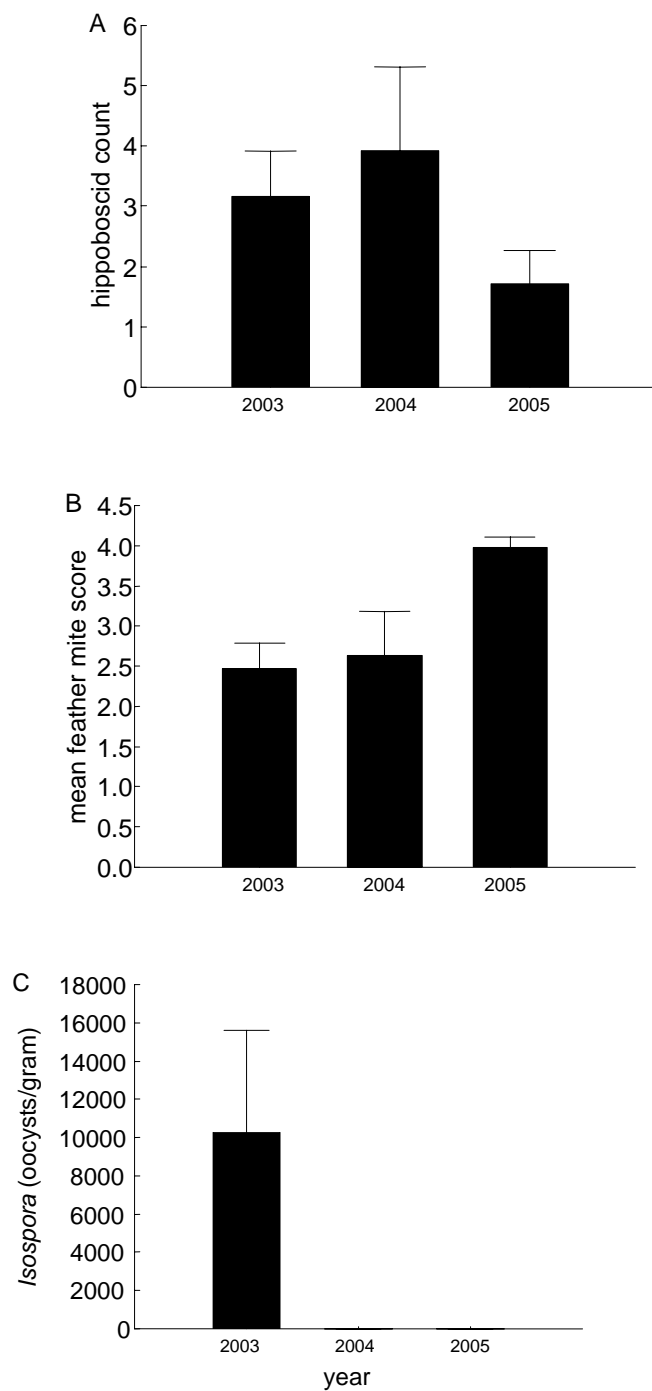


Figure 3.2 Difference in loads of feather mites (A), hippoboscids (B) and coccidia (C) loads between the three years following the 2002 population crash.

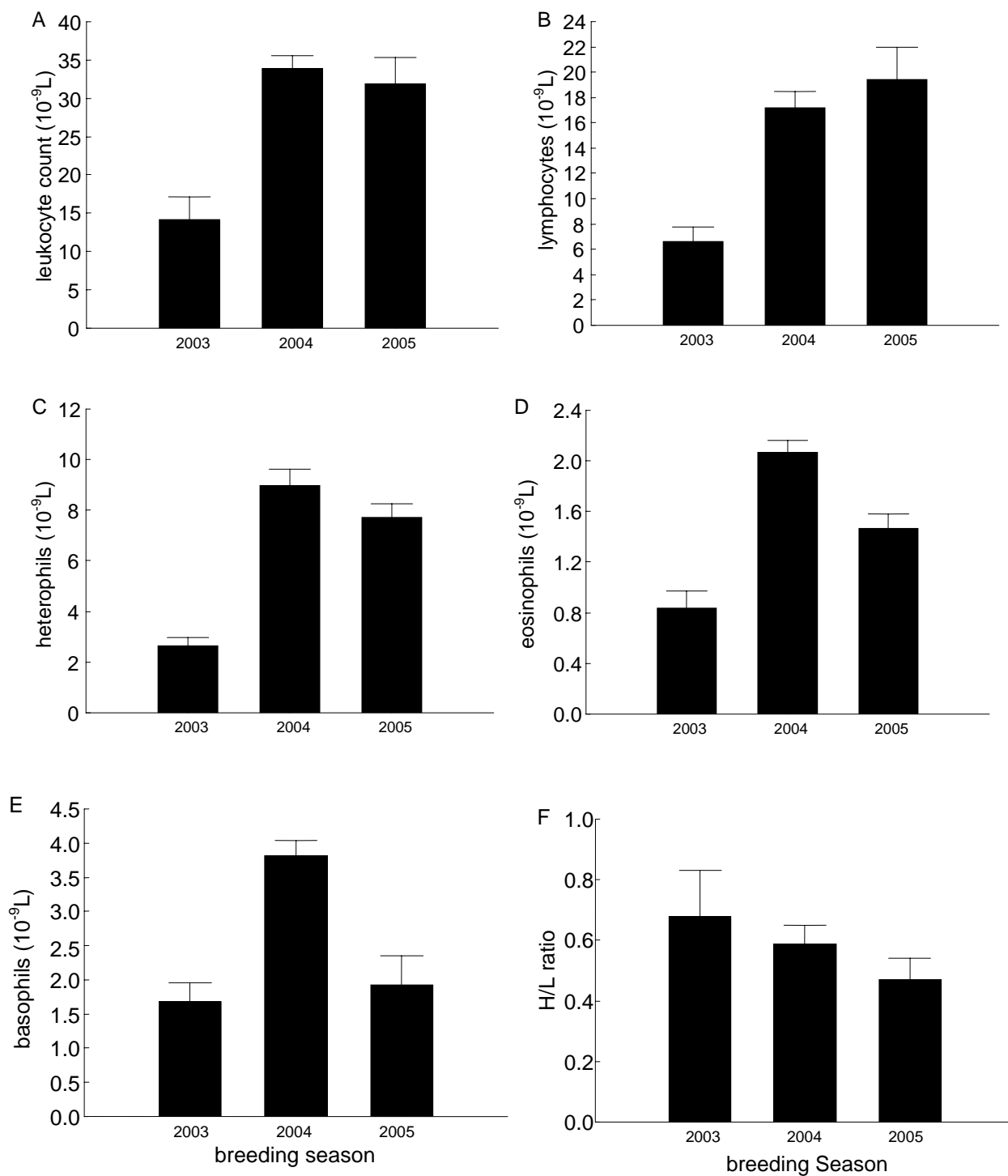


Figure 3.3 Nestling blood profiles showing differences between nestlings from the three breeding seasons following the population crash in leukocyte counts (A) and lymphocyte (B), heterophil (C), eosinophil (D) and basophil (E) counts and H/L ratios (F)

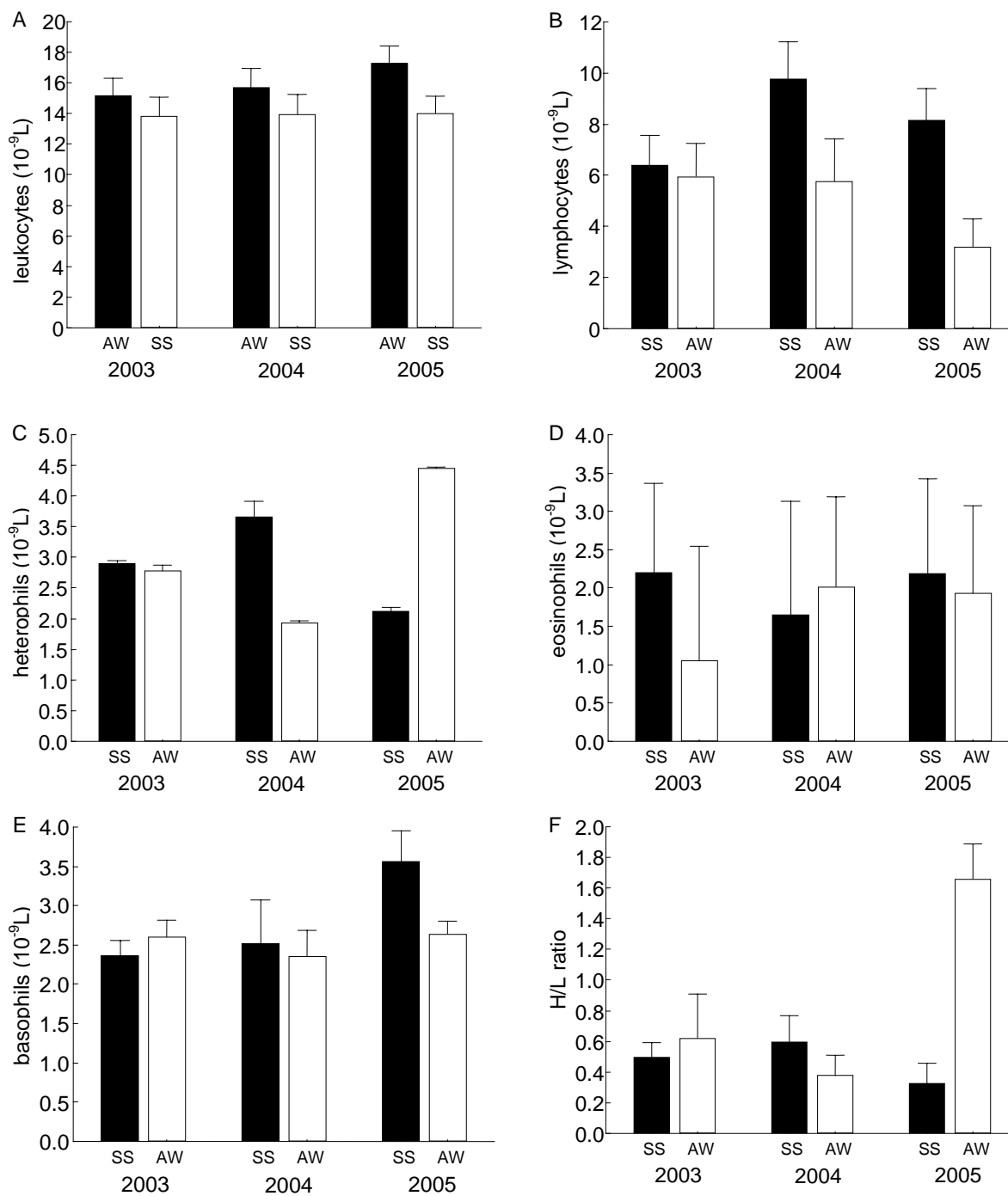


Figure 3.4 Adult blood profiles showing relationships between leukocyte counts (A) in autumn/winter (AW: closed bars) and spring/summer (SS: open bars) for the three years following the 2002 population crash females; (B) Lymphocyte, (C) heterophil, (D) eosinophil and (E) basophil counts during both seasons for the three years following the crash and (F) H/L ratio for both seasons for all three years.

3.5 Discussion

When I tracked the recovery of an isolated population of saddleback for three years following a disease outbreak, I found no significant change in reproductive success, despite a rapid increase in population density following the crash. Clutch size, nesting success and nestling mass were similar for all three breeding seasons, suggesting there was little effect of increasing density on reproductive performance, at least in the range of densities I observed. In contrast, I did find evidence to suggest that the increasing density over the three years of the study may have led to increasing risk of parasitism and negative effects on the immune system of saddlebacks. Although coccidia oocysts in faecal samples declined in the second and third years post-crash and there was little difference in the number of hippoboscids between years, I found a significant increase in feather mite numbers as the population recovered and density increased three-fold. I found evidence to suggest that fledgling survival was slightly lower, but not significantly so, for fledglings from the second breeding season compared to the first, with 31% individuals being sighted again in the following two years after the second breeding season compared to 51% from the first breeding season. As the population recovered to a high density (approx. 158 individuals), there was a significant increase in H/L ratio and a significant decrease in the number of lymphocytes for the autumn/winter period and a significant increase in basophil numbers in the spring/summer period for adult saddleback, indicating increasing stress levels and possible immunosuppression as the population density increases.

One possible interpretation for the lack of coccidial parasites isolated from faecal samples collected over the second and third years post-crash could be that this population has developed immunity to the parasite. Alternatively, it may simply be that the low

population density post-crash prevented a high enough rate of transmission for the parasite to survive, as is the case with many species-specific parasites (Altizer *et al.* 2001; Cleaveland *et al.* 2002). The lack of any current infection together with the population's rapid recovery to above its pre-crash density could lead to a false sense of security that this population is no longer under the threat of disease. However, while this population may now have immunity to one species of coccidia, the devastating degree to which this parasite initially affected the population suggests it may be vulnerable to other parasites and diseases.

The density of infective parasite stages in the environment is strongly influenced by environmental conditions such as temperature and humidity which determine the survival and/or emergence of infective stages (Scott 1988). Thus, the low hippoboscids found on individuals for the three years post-crash compared to the high level of infestation at the time of the crash suggests the environmental conditions at the time of the crash favoured the emergence, maintenance of high numbers and rapid spread of hippoboscids and coccidia. A high population density would also directly facilitate rapid transmission of parasites and disease and support parasites that require a critical host population density to persist (Armbruster & Reed 2005). Additionally, the crash coincided with a reduction in natural water sources on the island due to drought conditions (pers. obs.) which would mean individuals were forced to congregate at the few stagnant water stations provided, further facilitating the transmission of disease between both individuals of the same species and between species (Dobson & Foufopoulos 2001). While saddleback and several other endemic bird species on the island are poor fliers, thus making immigration and emigration impossible without human intervention, other introduced and native species are readily able

to fly between the island and mainland. Thus the risk of the introduction of a new parasite exhibiting a wide host range is high (Altizer *et al.* 2001; Swinnerton *et al.* 2005).

Alternatively a lack of water could further stress individuals, increasing susceptibility to diseases already present but under control when individuals are in good condition. High population densities and environmental conditions which act in synergy to favour an explosion of parasitic organisms may increase the susceptibility of inbred host populations as such conditions may act to exacerbate the effects of inbreeding which will in turn further magnify the metabolic costs of exposure to environmental stress and competition, making individuals even more vulnerable to environmental stress and diseases and ultimately extinction (Gilpin & Soule 1986; Hoffmann & Parsons 1991; Keller & Waller 2002).

The high H/L ratio, a measure of physiological stress, was found to increase in adult saddlebacks as the population recovered and reached a high density. This suggests that this population may be reaching a threshold whereby individuals may not be able to cope with any additional stressors such as extreme weather conditions (e.g. drought) and/or the introduction of a novel parasite. Prolonged periods of physiological stress, as would be the case in crowded environments, are believed to have an influence on disease resistance by depleting lymphoid cells which play a key role in the cell-mediated and humoral immune responses (Wikelski & Cooke 2006). Stress, by definition, is an agent requiring continuous excess energy expenditure from an organism (Hoffman & Parsons 1991). The increased energy demands due to environmental stress may mean fewer resources are available to put into immune functioning (Gustafsson *et al.* 1994; Nordling *et al.* 1998). If bottlenecked populations suffering from inbreeding depression are already immunodeficient as speculated (Reid *et al.* 2003; Hawley *et al.* 2005; Whiteman *et al.*

2006; Hale & Briskie 2006), then further suppression of the immune system due to increased stress levels could jeopardise the survival of many endangered populations. That I found a significantly lower lymphocyte number in the autumn/winter period when this population reached high density supports these conjectures and indicates the population may have reached a threshold limit of stress at which it is at an increased risk of a disease outbreak. Further, these results suggest that the post-breeding autumn/winter period is a critical time in terms of the vulnerability to disease outbreaks. The concept that reproduction is costly is central to life-history theory (Williams 1966) and there is ample theoretical and empirical evidence demonstrating that a trade-off exists between reproduction and immune function (Gustafsson *et al.* 1994; Deerenberg *et al.* 1997; Nordling *et al.* 1998; Moreno *et al.* 1999). The ability to resist parasites is to some degree sacrificed in order to put more energy into producing and caring for young. Thus the timing of the initial population crash may have occurred after the breeding season because this is the time when the birds would have been under most stress from raising their broods.

In contrast to adult birds, blood parameters and growth of nestlings and fledglings were apparently unaffected by the increase in population density. Instead H/L ratio remained the same and leukocyte and lymphocyte counts increased suggesting no change in stress levels and higher immune function in the second and third breeding season when the population density was higher compared to the first breeding season following the crash. This would tend to suggest that the parent birds may still have been in poor condition during the first breeding season following the crash and those that survived the crash had possibly expended more resources into immune function at the cost of reproduction. This could also explain why there appeared to be no second broods during the initial breeding

season following the crash. In contrast, in the second and third breeding seasons the adult saddlebacks did not appear to have compromised the upbringing of their young and were possibly able to compensate for the adverse affects of a high population density that they themselves are unable to avoid.

I found significantly higher counts of basophils in males during year three, and while very little is known about the function of the avian basophil, there is evidence linking high numbers of basophils in the peripheral blood to increased stress levels (Maxwell 1993; Fudge 2000). Thus the higher basophil counts I observed in males may indicate elevated stress levels arising from a result of an increased workload. For example, at high densities food is unlikely to be as plentiful, thus requiring more time foraging in order to obtain sufficient food for the nestlings. It is well known that reproduction is a major ecological stress birds face (Williams 1966; Raberg *et al.* 1998; Shutler *et al.* 2004) however, the increased workload imposed on the adult birds due to a high number of competing individuals will mean that even less resources are available to put into immune function and thus the trade-off between reproduction and immunocompetence becomes even greater and ultimately comes at a greater cost.

Identifying susceptible populations and predicting where and when a problem is likely to arise is a crucial step in preventing disease outbreaks and extinctions and yet often the most difficult owing to the lack of baseline data for many endangered populations (Wikelski & Cooke 2006). Without baseline data it is very difficult to recognize any abnormal departures in the health of a population, which is why most diseases are discovered after epidemics have already spread through wild populations (Altizer *et al.* 2001). While high parasite burdens can indicate a decrease in immune function, using

current parasite loads alone is an unreliable measure of susceptibility since high parasite loads and/or disease often require specific environmental conditions and thus they can remain at low numbers within the host population and not become a problem until such conditions arise. One could argue then that it is not high population densities or inbreeding that is problematic but simply environmental conditions such as weather which cannot be controlled. However, while high densities or inbreeding alone may not be the direct cause of a disease outbreak, these two factors combined not only create a susceptible population but also the optimal conditions for maximum disease transmission rates should a pathogen be introduced or new conditions occur that favour a population explosion of an already existing parasite or disease (Altizer *et al.* 2001). Conservation managers and biologists need to work towards establishing a monitoring programme which would enable baseline data to be obtained thus facilitating faster detection of increasing stress levels and declining health (Wikelski *et al.* 2004). Basic measures of immune parameters and physiological stress can offer a meaningful measure of present stress by establishing cause and effect. Such measures also provide essential baseline data, and therefore aid in quicker identification of populations in most need of immediate attention (Wikelski & Cooke 2006). Such measures would also aid in determining which individuals to use to found new populations, how large islands or remnant habitats need to be, and their suitability to prevent overcrowding and maintain the evolutionary potential of the species in question.

I found evidence to suggest high population density is a factor increasing stress levels which can ultimately lead to disease outbreaks in endangered populations, particularly those exhibiting reduced genetic diversity as a result of passing through a severe population bottleneck as happened to the South Island saddleback. The vast number

of endangered species that exist in degraded habitats and exhibit low genetic diversity, together with the rate and frequency at which environmental change is occurring and the rapidly increasing geographical range of many pathogens, emphasise the need to understand the relationship between stress, both genetic and environmental, and disease.

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Chapter 4

Haematological parameters and fluctuating asymmetry provide evidence for increased stress in bottlenecked populations of the endangered New Zealand saddleback

4.1 Abstract

Endangered species passing through severe bottlenecks generally exhibit a loss of genetic heterozygosity and increased inbreeding, which could lead to higher levels of stress and increased vulnerability to parasitic infection and adverse environmental conditions. To determine whether bottlenecked populations are more stressed, and if the level of stress varies with the severity of the bottleneck size each population passed through, I compared levels of fluctuating asymmetry (FA) in three morphological traits (tarsus, nares and wattle size), blood parameters, and parasite loads among 12 bottlenecked populations of saddleback (*Philesturnus carunculatus*). I found that populations founded by fewer individuals had significantly higher loads of ectoparasitic feather mites and H/L ratios and lower numbers of circulating lymphocytes. These patterns are suggestive of an increased level of stress and a lower level of immune function in individuals from populations passing through the most severe bottlenecks. It was evident from these results that a founder number of at least 90 individuals is required to minimise the deleterious effects of bottlenecks on immune function. In contrast, I found no relationship between measures of FA and bottleneck size. However, populations with higher tarsus asymmetry had higher hippoboscids fly counts and lower coccidia burdens, suggesting a trade-off between growth and immune response to parasites. I also identified island size as an additional stressor, with populations on smaller islands exhibiting higher H/L ratios and heterophil numbers compared to those on larger islands. My results indicate that small founder number and island size are key factors contributing to elevated stress levels in endangered populations, increasing susceptibility to parasitic outbreaks.

4.2 Introduction

Stress is defined as any factor that reduces fitness of a population or causes a potentially adverse change in an organism or biological system (Lincoln *et al.* 1998). In general, stress increases energy demand and can place an organism at a disadvantage, and ultimately threaten its survival (Parsons 1992). Most endangered species are probably subject to some form of environmental stress (e.g. reduction in habitat quality due to human activities) but in addition, they frequently suffer from reduced genetic diversity, which may in itself present a significant form of stress. Population bottlenecks, whether through founder events or population decline, can result in loss of genetic diversity and an increase in the rate of inbreeding as survivors are forced to mate with close relatives (Keller 1998; Frankham *et al.* 2002). This in turn may lead to inbreeding depression, possibly lowering stress tolerance levels and directly threatening the survival of the population (Frankham 1995; Frankham 2005).

One increased environmental stressor that endangered species face is parasites and disease (O'Brien & Evermann 1988; Dobson & Foufopoulos 2001; Friend *et al.* 2001; Spielman *et al.* 2004). Although all species must cope with various parasites and diseases, population bottlenecks and inbreeding may render a population more vulnerable to some parasites and diseases as their immune systems may be less adaptable or defective as a result of reduced genetic variation (O'Brien & Evermann 1988; Spielman *et al.* 2004). Indeed, inbreeding depression has been recognised as a key factor contributing to increased disease susceptibility and disease outbreaks in endangered wild populations and recent experimental studies suggest inbred populations exhibit lower levels of immunocompetence (Reid *et al.* 2003; Hawley *et al.* 2005; Hale & Briskie 2006). In addition to the negative

effects the loss of genetic diversity may have on the immune system, it is widely speculated that severe stress, such as reduced genetic variation, applied at certain critical stages of development can cause abnormal phenotypes to be produced, disrupting developmental homeostasis and leading to developmental asymmetries and abnormalities (Hoffmann & Parsons 1991). As such, fluctuating asymmetry (FA), a measure of developmental instability (DI), has often been proposed as an indirect estimate of inbreeding, genetic diversity and population fitness in endangered populations since it arises from environmental and genetic stress occurring during ontogeny (Polak 1997). Additionally, because asymmetry is believed to reflect a lack of perfect adaptation to the environment (Møller 2006), it is thought FA may reflect the adaptation and evolution of populations as well as the genetic processes associated with extinction (Leary & Allendorf 1989). While evidence is growing to suggest population bottlenecks disrupt immune function, it remains unclear what minimum founder number is required to avoid these detrimental effects and if stress arising from population bottlenecks also manifests in the form of developmental asymmetries.

I measured fluctuating asymmetry, the ratio of heterophils to lymphocytes (H/L ratio) and compared counts of leukocytes and external, blood and gastrointestinal parasite loads in the saddleback (*Philesturnus carunculatus*), a rare endemic bird species that has suffered extensive decline since human settlement of New Zealand and the subsequent introduction of a number of exotic mammalian predators. My goal was to determine if more severely bottlenecked populations exhibit higher levels of stress, specifically whether they exhibited higher levels of fluctuating asymmetry and higher H/L ratios, and to determine any effects of population bottlenecks on immune function and parasite loads.

4.3 Materials and methods

4.3.1 Study populations

The saddleback is one of three species in the unique wattlebird family Callaeidae. It is a medium-sized passerine (70-90 g) that feeds on invertebrates and fruits on the forest floor (Heather & Robertson 2000). There are two distinct geographical subspecies of the saddleback: *Philesturnus c. carunculatus* in the North Island and *Philesturnus c. rufusater* in South and Stewart islands. Both were once widespread but were reduced to two single offshore island populations (one population of each subspecies) following human settlement and the subsequent introduction of mammalian predators. The North Island saddleback declined to only ~500 individuals on Hen Island, while the South Island saddleback declined to only 36 survivors rescued from Big South Cape Island before this population was exterminated. Following a series of translocations, both subspecies now thrive on numerous predator-free offshore islands around New Zealand. The present study includes six North Island saddleback populations: Hen (the source population, 484 ha), Cuvier (170 ha), Lady Alice (155 ha), Tiritiri Matangi (197 ha), Mokoia (135 ha) and Kapiti (1965 ha) and six South Island saddleback populations: Big (23 ha), Kaimohu (11 ha), Putauhinu (141 ha), Breaksea (170 ha), Motuara (59 ha) and Ulva (270 ha). For most of these populations, individuals have been translocated from populations that were themselves previously translocated (Fig. 4.1) and it is likely that the original source populations for both subspecies (Hen and Big South Cape islands) were themselves bottlenecked prior to any subsequent translocations (Hooson & Jamieson 2003). Thus most existing populations are serially bottlenecked to some degree potentially causing serial reduction in genetic diversity. While there is information on the number of founders and

their source for each translocation event (Fig. 4.1), detailed post-translocation information is generally lacking and hence I have limited information on survival or rate of increase of each population subsequent to translocation. However, most successful translocations of saddlebacks are typified by high survival of the translocated birds and a rapid increase in numbers (Armstrong *et al.* 2002; Hooson & Jamieson 2003; Taylor *et al.* 2005) and I assumed for this study that the number of birds translocated was a suitable estimate of population bottleneck size.

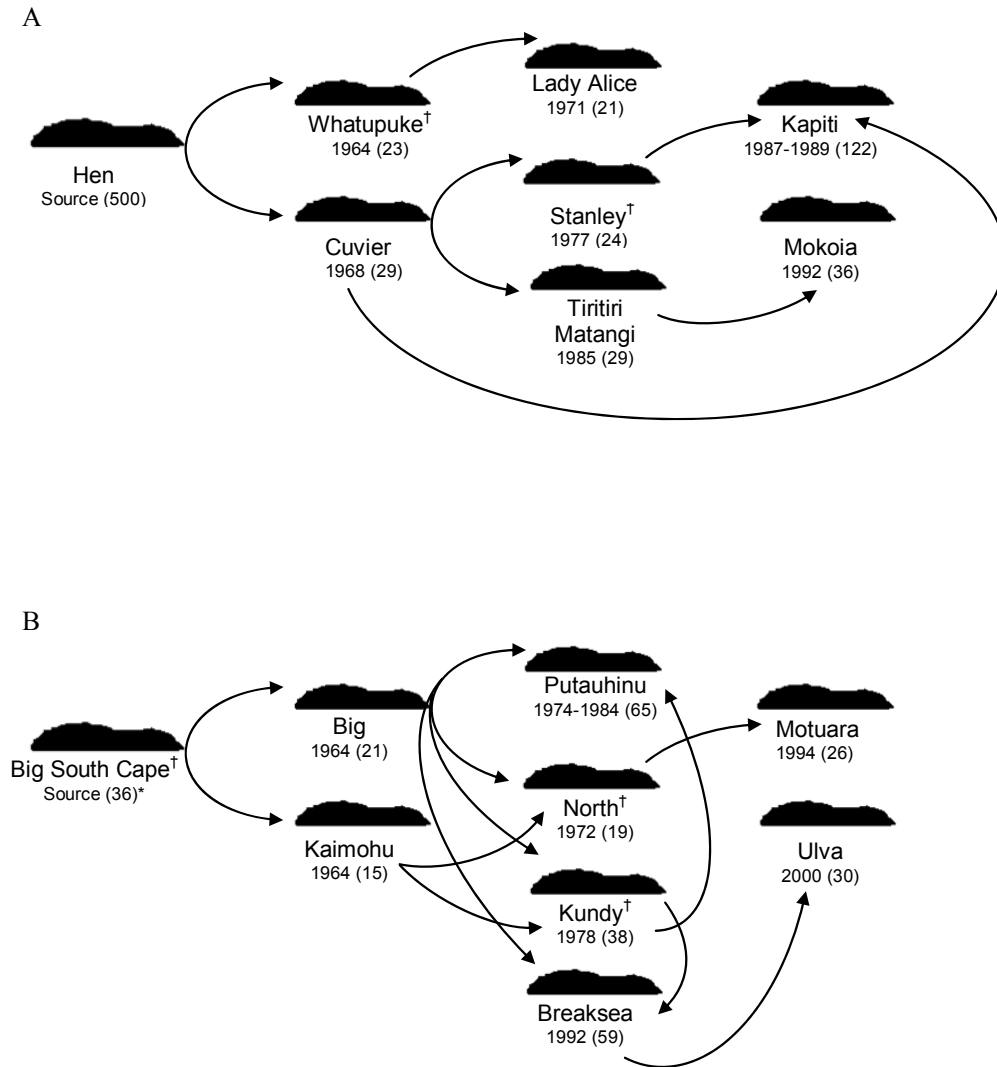


Figure 4.1 Pattern of successful translocation of saddlebacks starting with the two source islands: Hen Island for the North Island saddleback and Big South Cape for the South Island saddleback. The date of the transfer is given along with the number of individuals transferred in parentheses. Arrows indicate the sequence of transfers and show the founder source for each island. The symbol [†] denotes islands that were not sampled in this study and * indicates saddleback are no longer present on Big South Cape due to the removal of all surviving individuals following and invasion by ship rats (*Rattus rattus*).

4.3.2 Parasite loads

Between October 2002 and August 2006 I caught 290 adult and 153 sub-adult (> 1 but less than 2 years old) saddlebacks using mist-nets. Birds were readily aged and sexed from plumage and size characteristics. All birds were colour banded for individual identification and an estimate of feather mite density was obtained for each individual by examining the primary feathers of the left wing. Feather mite density was given a categorical score from 0 – 5 where: 0 = no feather-mites; 1 = 0-10; 2 = 10-100; 3 = 100-1000; 4 = 1000-10000 and 5 = 10000+ feather-mites. The number of hippoboscids flies (*Ornithomya* spp. and *Ornithoica* spp.) seen on or flying off the bird were also counted on each bird. Faecal samples were collected from the holding bags (the birds normally defecated whilst being held in the cloth holding bags) for analysis for gastrointestinal nematodes and the protozoan parasite coccidia. Faecal egg counts to estimate coccidia and nematode burdens were carried out at a commercial laboratory using standard faecal flotation techniques (New Zealand Veterinary Pathology Ltd, Hamilton).

4.3.3 Fluctuating asymmetry

Three traits were selected for the study of asymmetry: tarsus length, nares length (from distal end of nasal opening on bill to upper mandible tip of bill) and maximum wattle length. Two left and right measurements of each trait were made to the nearest 0.01 mm with slide callipers, which were reset to zero between measurements. The sequence of measurement was always right-left-right-left to ensure some independence of measurements. All measurements in this study were carried out by myself.

Fluctuating asymmetry was analyzed as outlined in the steps given by Palmer and Strobeck (2003); a brief outline is given as follows. A Spearman's rank correlation was first

carried out to assess the degree to which, if at all, FA depended on trait size. To determine if the traits measured ideal or “true” FA and not other forms of asymmetry (directional symmetry (DA) or antisymmetry), I analysed measures of skew and kurtosis to determine any significant antisymmetry and DA (as mean (R-L)/SE(R-L)) using the table provided by Palmer & Strobeck (1986). Because the degree of fluctuating asymmetry measured is often very small (often around 1%, [Palmer 1994]), it is necessary to determine if the between-sides differences are significantly greater than measurement error. This was achieved by carrying out a simple sides x individual ANOVA.

I determined four FA indices: FA1, FA4a and FA10a (FA10a describes the average difference between sides after measurement error has been factored out) to give an overall measure of FA (Palmer & Strobeck 1986; Palmer & Strobeck 2003). Seven of the island populations showed significant directional asymmetry after sequential Bonferroni correction for multiple tests. However, only three of these populations had $DA > FA4a$ (see Palmer & Strobeck (2003) for general rule of thumb for significant measures of DA). Tiritiri Matangi Island males (nares), Ulva Island females (nares) and Putauhinu Island females (tarsus) were identified as the main contributors of the DA in these three groups. Thus, while it seems unlikely that I would measure sexes differently, I nonetheless excluded these subgroups from further analyses. Six populations for tarsus and five populations for nares were significant for measurement error (Table 4.1).

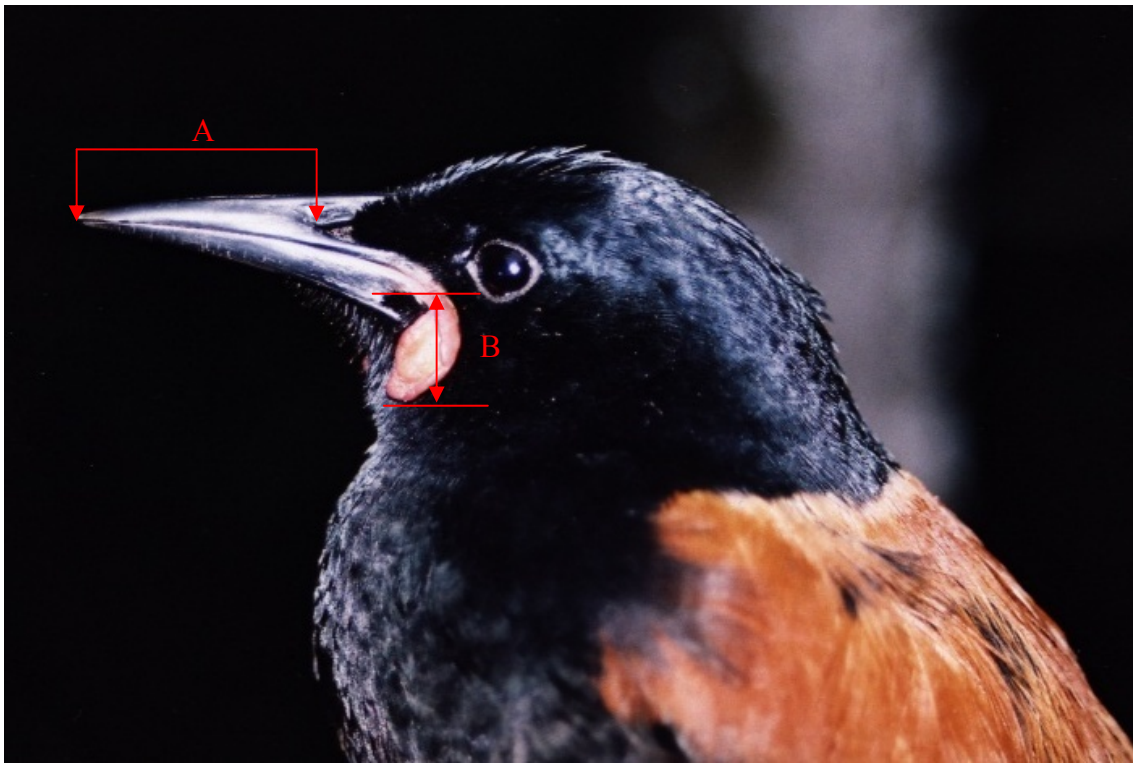


Figure 4.2 Photo showing the positioning used for the nare (A) and wattle (B) measurements.

4.3.4 Blood parameters

Approximately 200 μL of blood was obtained from the right wing of each individual via brachial venipuncture. To measure leukocyte parameters, a drop of blood was smeared onto a glass slide, air dried, fixed in methanol, and then stained using a modified May-Grünwald Giemsa staining method (Lucas & Jamroz 1961). Blood smears were then viewed under a light microscope and the following measurements taken: (1) total leukocyte number (referred to here as leukocyte count), which can give an indication of the overall health of the individual at the time of sampling and is calculated by counting all leukocytes in ten consecutive fields of view (at 400 X magnification) which are then averaged to give an estimate for each individual (Walberg 2001); and (2) a leukocyte differential which is obtained by examining each blood smear under oil immersion (1000 X magnification)

using a cross-sectional (down-across-up-across-down etc.) scanning method and determining the relative frequency of the five different types of leukocyte (lymphocytes, heterophils, basophils, eosinophils and monocytes) for a total of 100 leukocytes. The heterophil/lymphocyte ratio (H/L) was calculated by dividing the number of heterophils by the number of lymphocytes. Finally, the blood smear was scanned for three minutes using the same cross-sectional method to detect any blood borne parasites.

4.3.5 Statistical analyses

I performed all statistical analyses in Statistica 6 (StatsSoft, Inc.). Any significant outliers for either FA (due to measurement errors or aberrant individuals) or the leukocyte data were identified using Grubbs analysis (Sokal & Rohlf 1995) and removed following Bonferroni Sequential correction for multiple tests. Data transformations were applied to the variables that did not conform to normality. I used ANOVAs, or the non-parametric Kruskal-Wallis test if the data could not be normalised, to determine if there were any significant differences between sexes in FA and leukocyte parameters between the islands. I then carried out Pearson correlation matrices and backward step-wise multiple regression models to determine if trait asymmetry, leukocyte counts, the counts of lymphocytes and heterophils, and H/L ratio were related to either island founder number, the number of population bottlenecks, island size or saddleback population density.

4.4 Results

4.4.1 Parasite loads and bottleneck size

All 12 saddleback populations in this study were found to harbour feather mites (Table 4.2) and there was a significant inverse relationship between founder size and mean feather mite score, with individuals having on average more feather mites on islands that were founded by fewer individuals ($r = -0.78$, $N = 12$, $P < 0.01$: Fig. 4.2a, Table 4.2). Island size also correlated significantly with mean feather mite score, with higher feather mite loads in saddleback populations on smaller islands ($r = -0.77$, $N = 12$, $P < 0.01$: Fig 4.2b, Table 4.2). In contrast, feather mite load was not related to the number of bottlenecks the population had been through or saddleback density on the islands and when I performed a backward step-wise generalised regression model, only founder number was significantly related to feather mite number ($F_{1,10} = 15.35$, $r = 0.78$, $P < 0.01$). I found no relationship between founder number, the number of bottleneck events, island size or density of birds per hectare and either hippoboscids or coccidia loads (Pearson correlation coefficients, all $P > 0.05$: Table 4.2). I detected no blood parasites from the blood smears of any saddleback from any population.

4.4.2 Fluctuating asymmetry and bottleneck size

There was no significant difference between the sexes in levels of FA, and this pattern held whether the three traits were combined or analysed separately (GLM, P all > 0.05).

I found no significant relationship between founder number, the number of population bottlenecks, island size, the number of birds per hectare and nares or wattles FA1 or FA10a (Pearson correlation coefficients, all $P > 0.05$: Table 4.1). Similarly there was also no

relationship between founder number and the number of bottlenecks and FA1 or FA10 tarsus (Pearson correlation coefficients, $P > 0.05$: Table 4.1). I did however find a significant correlation between island size and tarsus FA1 ($r = -0.58$, $N = 12$, $P = 0.045$: Fig. 4.3a), with populations from smaller islands exhibiting larger tarsus asymmetry than those on larger islands. Additionally there was a significant correlation between the number of birds per hectare and tarsus FA1, with populations from islands with higher saddleback densities exhibiting higher levels of tarsus asymmetry ($r = 0.60$, $N = 12$, $P < 0.05$: Fig. 4.3b). However, following a backward step-wise generalised regression analysis only island size was included in the final model as a significant predictor of tarsus FA1 ($F_{1,10} = 5.20$, $r = 0.58$, $P = 0.046$). In contrast neither island size nor the numbers of birds per hectare were related to tarsus FA10a (Pearson correlation coefficients, all $P > 0.05$).

Fluctuating asymmetry and parasite loads

There was no significant relationship between any measures of asymmetry and feather mite score (Pearson correlation coefficient $P > 0.05$). However, there was a significant correlation between mean hippoboscid load and tarsus FA1 ($r = 0.71$, $N = 11$, $P < 0.05$) and between mean hippoboscid load and mean coccidia oocyst count and tarsus FA10a ($r = 0.61$, $N = 11$, $P < 0.05$ and $r = -0.65$, $N = 11$, $P < 0.05$, respectively: Fig. 4.3c-d). Tarsus FA10a increased with increasing hippoboscid loads and decreasing coccidia loads. When I carried out a multiple regression backward stepwise model using tarsus FA10a as the dependent variable and feather mite score, hippoboscid and coccidia counts as the regressors, both hippoboscid and coccidia counts were included in the final model and both were significant predictors of tarsus FA10a (hippoboscid: $F_{1,8} = 6.84$, $r = 0.52$, $P < 0.05$; feather mite: $F_{1,8} = 8.06$, $r = -0.57$, $P < 0.05$). Conversely, there was no relationship

between nares and wattle FA1 and FA10a and either hippoboscids or coccidia loads (Pearson correlation coefficients, all $P > 0.05$)

4.4.3 Blood parameters and bottleneck size

Although I found a significant difference between islands for counts of leukocytes ($F_{11,429} = 6.59$, $P < 0.001$), lymphocytes ($F_{11,428} = 17.45$, $P < 0.001$), heterophils ($F_{11,425} = 10.73$, $P < 0.001$), eosinophils ($F_{11,421} = 6.27$, $P < 0.001$) and basophils ($F_{11,428} = 18.31$, $P < 0.001$), and H/L ratios ($F_{11,425} = 17.84$, $P < 0.001$), none of the white blood cell parameters correlated with the number of bottlenecks (Pearson correlation coefficients, all $P > 0.05$). and only H/L ratio and lymphocytes correlated with founder number ($r = -0.59$, $N = 12$, $P < 0.05$ and $r = 0.68$, $N = 12$, $P < 0.05$, respectively: Fig. 4.4a,b) Additionally, I found a significant correlation between island size and both H/L ratio and heterophil counts (Pearson correlation coefficients, $r = -0.73$, $N = 12$, $P < 0.001$ and $r = -0.64$, $N = 12$, $P < 0.05$, respectively: Fig. 4.4c,d) and between H/L ratio and the number of saddlebacks per hectare ($r = 0.59$, $N = 12$, $P < 0.05$: Fig. 4.4e). Because of the inter-correlation between H/L ratio and founder number, island size and saddlebacks per hectare, I carried out a backward stepwise general regression model to determine which, if any, of the variables predicted H/L ratio better than the others. Island size was the only variable to be retained in the stepwise model and was highly significant ($F_{1,10} = 11.49$, $r = 0.73$, $P < 0.01$). No other white blood cell parameters were correlated with island size or saddlebacks per hectare (Pearson correlation coefficients, all $P > 0.05$: Table 4.3). This was confirmed by carrying out backward stepwise general regression models on each of the specific blood parameters using founder number, island size and saddlebacks per hectare as the independent variables (GRM, all $P > 0.05$).

Table 4.1 Difference in fluctuating asymmetry parameters between North and South Island saddleback populations.

A	Trait size ± SE			Spearman ^a	(R-L) ^b			FA Indices ^c			ME3 ^d	DA ^e
Trait/population	N		ρ ^a	Mean ± SE	Skew	Kurtosis	FA1	FA4a	FA10a	%	Ts	
Tarsus												
Hen	15	42.24 ± 0.32	0.32	-0.01 ± 0.09	-1.45	3.67	0.236	0.284	0.259	16.26	-0.08	
Cuvier	41	39.74 ± 0.26	0.05	0.08 ± 0.05	-0.67	1.30	0.246	0.245	0.209	26.26	1.69	
Lady Alice	30	40.90 ± 0.29	0.10	-0.01 ± 0.04	0.18	0.96	0.158	0.157	0.038	94.11	-0.25	
Tiritiri Matangi	64	39.54 ± 0.22	0.16	0.04 ± 0.04	0.10	-0.23	0.239	0.236	0.203	25.15	1.00	
Mokoia	24	40.08 ± 0.31	0.10	-0.00 ± 0.06	-1.14	1.30	0.237	0.235	0.227	16.03	-0.02	
Kapiti	28	39.68 ± 0.29	-0.28	0.00 ± 0.04	-0.67	0.17	0.155	0.177	0.120	53.18	0.07	
Big	35	39.55 ± 0.27	0.12	-0.23 ± 0.05	0.34	-0.48	0.304	0.231	0.178	41.76	-4.78	
Kaimohu	16	40.26 ± 0.49	-0.04	-0.17 ± 0.07	-0.05	1.86	0.248	0.217	0.152	51.01	-2.50	
Putauhinu	7	42.59 ± 0.37	0.03	-0.18 ± 0.09	-1.27	1.54	0.199	0.196	0.121	62.56	-1.93	
Breaksea	24	41.74 ± 0.31	-0.29	0.04 ± 0.05	0.09	0.36	0.203	0.207	0.147	49.54	0.60	
Ulva	22	40.92 ± 0.36	0.23	-0.06 ± 0.04	-0.15	1.28	0.135	0.157	0.120	40.34	-1.40	
Motuara	139	41.68 ± 0.13	0.03	-0.14 ± 0.03	0.27	0.37	0.336	0.320	0.281	22.23	-4.24	
Nares												
Hen	15	21.42 ± 0.18	0.33	-0.15 ± 0.06	0.34	0.57	0.217	0.173	0.126	47.56	-2.73	
Cuvier	43	21.73 ± 0.16	-0.15	-0.17 ± 0.04	-0.11	-0.26	0.239	0.194	0.150	40.69	-4.68	
Lady Alice	29	21.66 ± 0.17	-0.01	0.03 ± 0.04	-1.50	2.82	0.139	0.155	0.117	42.28	0.72	
Tiritiri Matangi	36	20.99 ± 0.13	-0.12	-0.09 ± 0.04	0.42	0.43	0.199	0.177	0.152	27.84	-2.54	
Mokoia	24	20.61 ± 0.13	0.06	-0.11 ± 0.06	0.63	0.26	0.246	0.219	0.172	38.22	-1.98	
Kapiti	26	21.73 ± 0.13	-0.41	-0.11 ± 0.04	-1.01	1.06	0.166	0.179	0.159	19.75	-2.57	
Big	35	22.18 ± 0.24	-0.00	0.08 ± 0.03	-0.44	1.65	0.156	0.156	0.106	52.74	2.46	
Kaimohu	16	21.24 ± 0.25	0.18	0.08 ± 0.05	0.20	-0.68	0.162	0.156	0.113	47.34	1.65	
Putauhinu	15	22.81 ± 0.41	0.29	0.08 ± 0.04	0.36	-0.50	0.137	0.136	0.073	70.89	1.82	
Breaksea	24	22.37 ± 0.28	0.04	-0.04 ± 0.05	-0.71	-0.02	0.193	0.192	0.173	17.16	-0.84	
Ulva	7	22.83 ± 0.57	-0.02	-0.16 ± 0.10	1.62	1.88	0.164	0.201	0.155	40.23	-1.65	
Motuara	137	21.75 ± 0.10	-0.00	-0.05 ± 0.02	0.00	-0.36	0.194	0.187	0.147	38.72	-2.20	
Wattles												
Hen	15	10.56 ± 0.31	0.18	-0.44 ± 0.14	-0.95	0.70	0.507	0.423	0.412	4.73	-3.23	
Cuvier	29	8.62 ± 0.25	0.28	0.04 ± 0.09	-0.20	-0.41	0.489	0.387	0.381	10.72	3.42	
Lady Alice	26	9.28 ± 0.29	0.08	0.35 ± 0.11	-0.55	0.62	0.535	0.460	0.449	4.86	3.08	
Tiritiri Matangi	49	8.19 ± 0.24	0.14	-0.11 ± 0.10	0.64	0.89	0.519	0.536	0.472	5.55	-1.14	
Mokoia	21	9.17 ± 0.33	0.18	0.00 ± 0.12	-0.20	1.01	0.399	0.446	0.431	6.62	0.03	
Kapiti	24	8.57 ± 0.25	-0.18	0.10 ± 0.09	0.44	0.39	0.356	0.360	0.345	7.39	1.05	
Big	28	6.66 ± 0.26	0.28	-0.08 ± 0.12	-0.06	-0.62	0.486	0.486	0.469	6.34	-0.66	
Kaimohu	12	6.88 ± 0.39	0.14	-0.04 ± 0.10	-0.69	-0.38	0.262	0.274	0.246	18.44	-0.35	
Putauhinu	12	7.57 ± 0.41	-0.25	0.14 ± 0.13	0.09	-1.33	0.378	0.362	0.333	15.71	1.10	
Breaksea	14	5.68 ± 0.34	0.41	-0.08 ± 0.15	-1.50	3.68	0.384	0.460	0.451	3.96	-0.50	
Ulva	12	7.97 ± 0.31	0.55	-0.16 ± 0.23	1.20	0.99	0.632	0.639	0.631	2.35	-0.68	
Motuara	45	8.85 ± 0.16	-0.35	-0.04 ± 0.08	-0.39	0.11	0.429	0.450	0.432	8.43	-0.43	

^a measure of dependency of FA on trait size^b Skew and kurtosis provide measure of antisymmetry^c FA1 = $|R-L|$; FA4a = $0.798\sqrt{N}\cdot SE^2$; FA10a = $0.798\sqrt{10}\cdot \sigma_1^2$ (see Palmer & Strobeck 2003, 1986; Palmer 1994 for full details)^d ME3 = $100 \cdot MS_m / MS_{interaction}$ (see Palmer & Strobeck 2003, 1986 and Palmer 1994 for full details)^e DA = directional asymmetry

Table 4.2 Parasite loads for the different saddleback populations. N is the number of birds sampled in each population.

Population	# of founders	Population density ^a	Parasite loads					
			Feather mite		Hippoboscid		<i>Isospora</i> sp	
			N	Mean Score	N	Mean count	N	oocysts/gram
Hen	500	1.01	15	0.60	15	0.07	13	1573
Cuvier	29	5.90	42	3.16	42	0.14	31	6072
Lady Alice	21	1.94	30	3.10	30	0.00	27	33668
Tiritiri Matangi	29	6.40	63	2.32	63	0.68	45	12326
Mokoia	36	1.48	21	1.76	21	0.48	10	260
Kapiti	122	0.05	28	1.21	27	0.07	11	849
Big	21	8.70	33	4.15	33	0.00	27	0.00
Kaimohu	15	2.72	16	4.00	16	0.00	16	353
Putauhinu	65	2.13	15	3.80	15	0.00	14	15207
Breaksea	59	2.35	22	3.05	22	0.00	13	20692
Ulva	26	0.11	18	3.28	17	0.00	0	*
Motuara	30	2.54	116	3.43	119	2.09	50	5128

^a Measured as saddlebacks per hectare, data provided by the Department of Conservation

Table 4.3 Mean leukocyte counts and differentials and H/L ratio for the different North and South Island saddleback populations.

Population	N	leukocyte count ($10^9/L$)	Leukocyte differentials ($10^9/L$)					Stress index
			Lymphocyte	Heterophil	Monocyte	Basophil	Eosinophil	H/L
Hen	13	29.27	20.00	2.30	0.28	2.03	2.30	0.13
Cuvier	42	11.65	3.40	2.82	0.29	2.31	1.49	0.98
Lady Alice	30	18.22	8.00	3.19	0.14	3.20	2.34	0.46
Tiritiri Matangi	58	22.34	11.80	2.24	1.23	2.64	2.67	0.25
Mokoia	25	21.02	11.55	3.60	0.36	3.04	1.18	0.37
Kapiti	21	14.01	7.99	0.83	0.53	2.02	0.99	0.11
Big	34	16.42	3.90	6.47	0.09	2.80	1.11	2.02
Kaimohu	16	18.95	4.98	3.30	0.60	4.21	2.88	0.86
Putauhinu	15	18.83	6.02	4.80	0.30	3.28	2.01	0.89
Breaksea	70	14.52	3.39	5.13	0.36	0.73	3.18	1.81
Ulva	18	16.02	7.75	2.17	0.76	0.99	2.38	0.37
Motuara	99	15.38	5.02	3.22	0.79	2.45	2.14	0.85

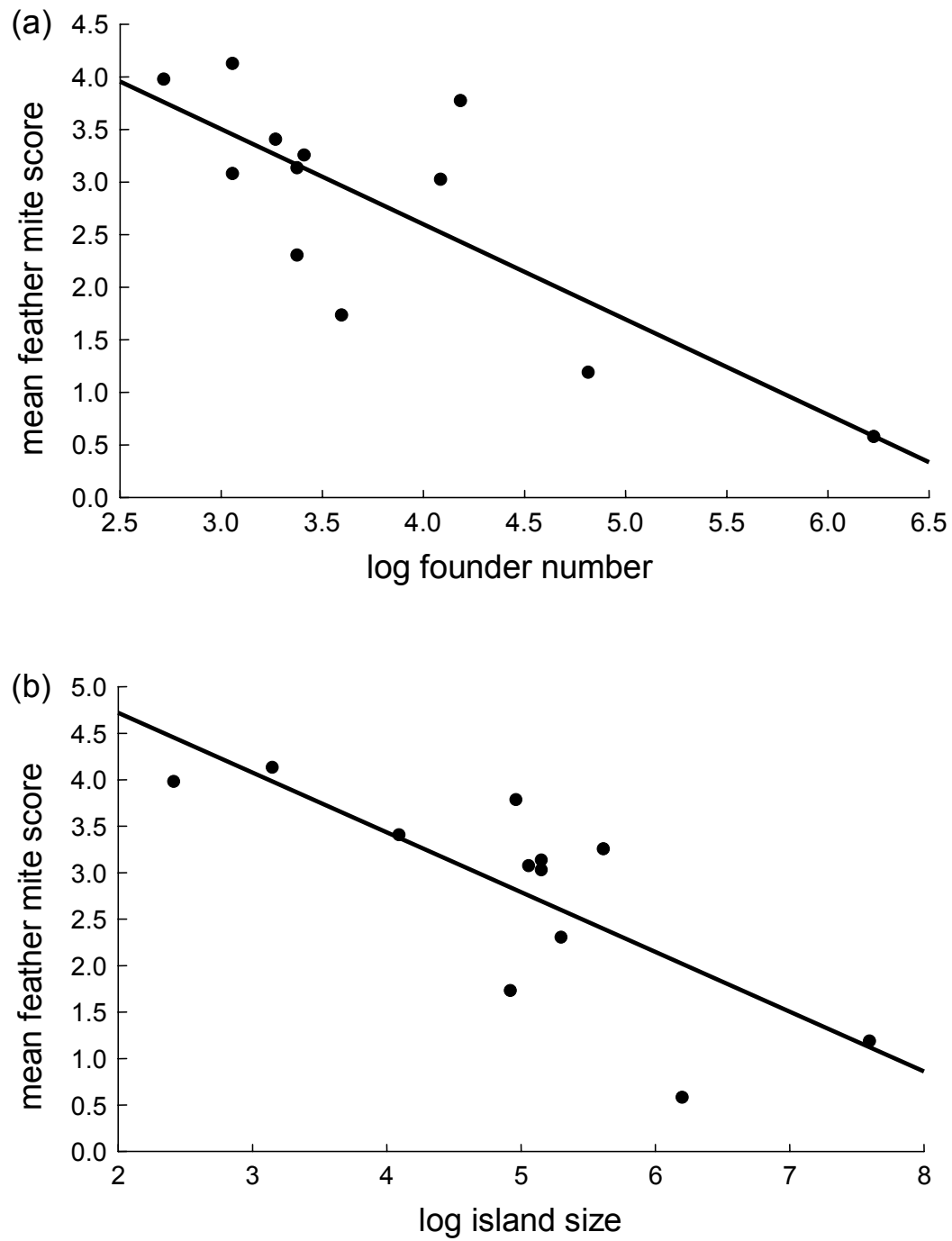


Figure 4.3 Relationship between mean feather mite score for each island and island founder number (a) and island size (b).

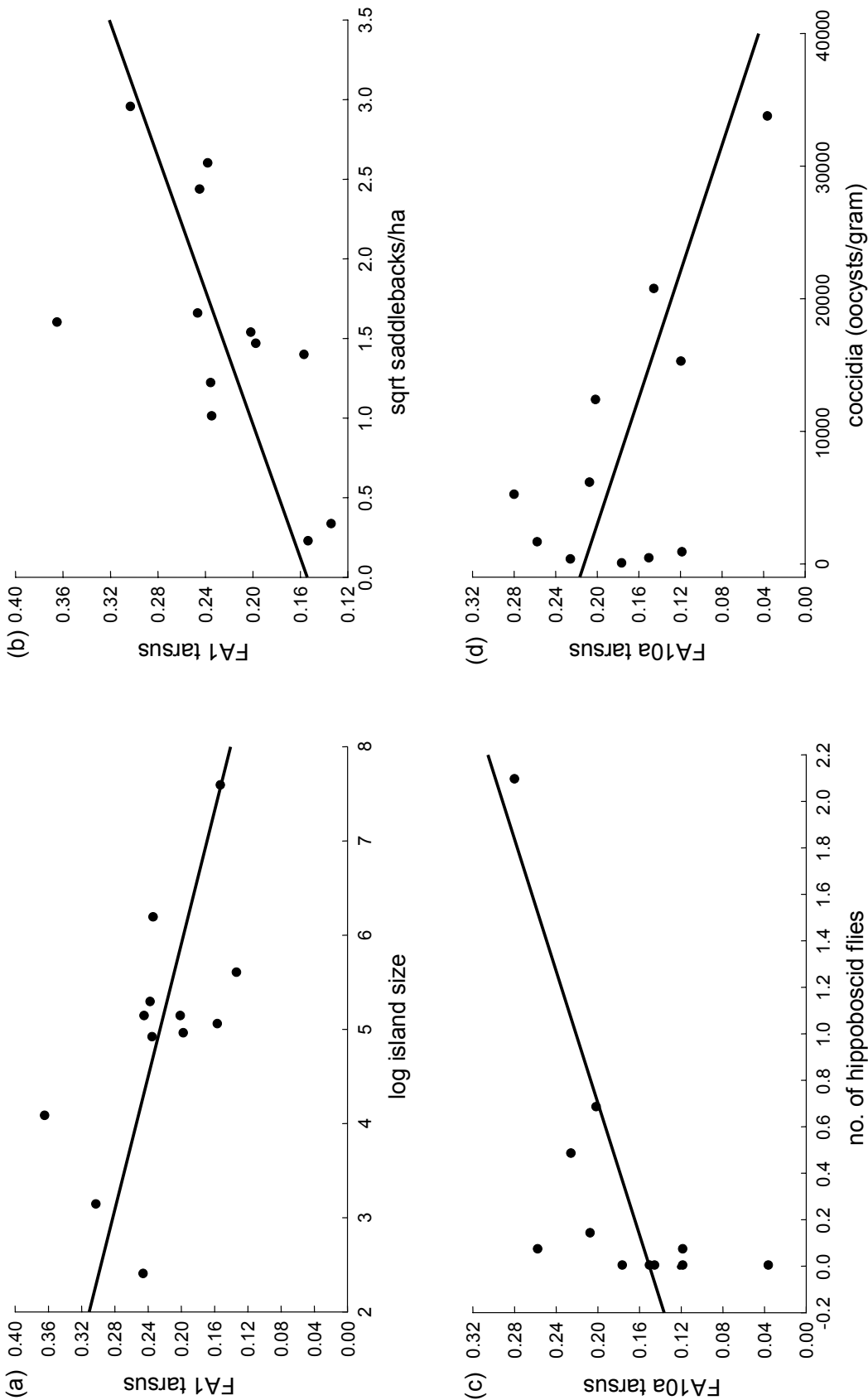


Figure 4.4 Relationship between island FA1 tarsus and island size (a) and saddlebacks per hectare (b) and between island FA10a and the mean number of hippoboscoid flies (c) and coccidia faecal egg counts (d).

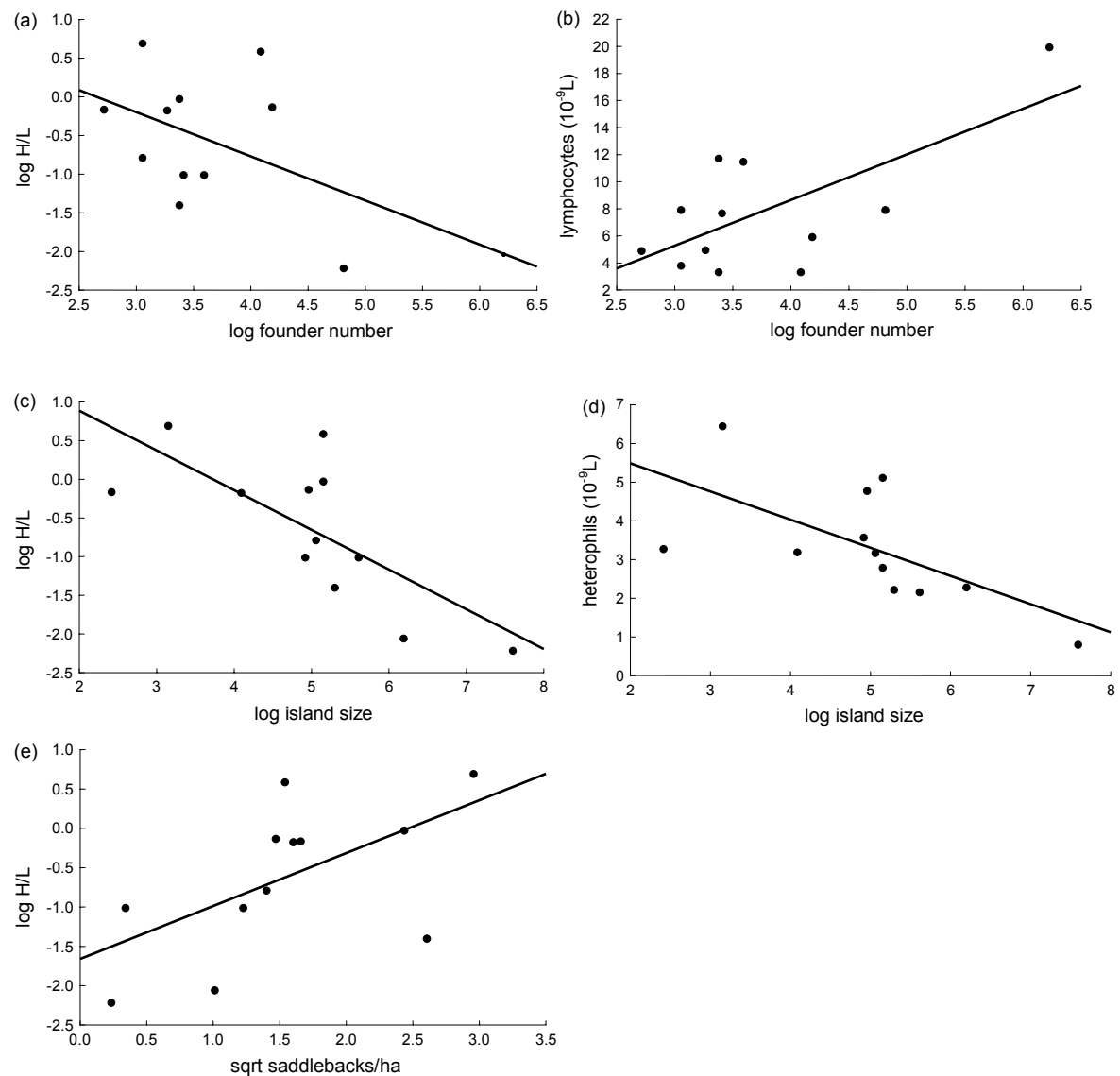


Figure 4.5 Relationship between island founder number and H/L ratio (a) and mean lymphocyte counts (b) and between island size and H/L ratio (c) and mean heterophil counts (d) and between the number of saddlebacks per hectare and H/L ratios (e).

4.5 Discussion

When I examined 12 populations of saddleback I found that populations that had gone through more severe bottlenecks had higher H/L ratios, lower numbers of circulating lymphocytes and higher feather mite loads. In addition to founder number, populations with higher saddleback densities also had higher H/L ratios and similarly populations from smaller islands showed higher H/L ratios and higher heterophil numbers and feather mite loads than those from larger islands. These results suggest elevated levels of stress and possible immunosuppression in populations that have gone through more severe bottlenecks, have a high density and in particular, exist on small islands. In contrast, when I examined fluctuating asymmetry in three bilateral characters among the 12 populations of saddlebacks, I found no relationship between founder number and the level of nares or wattle asymmetry. I did find a weak relationship between tarsus asymmetry (FA1) and founder number, however the relationship did not exist for FA10a where measurement error is factored out and hence I cannot rule out that the relationship is due solely to measurement error. Similarly I found a weak relationship between the number of saddlebacks per hectare and tarsus (FA1) asymmetry, but again the relationship was weak and it did not hold true for FA10a.

My general regression model analyses revealed that while small founder numbers and larger densities of saddlebacks were related to increases in H/L ratio, island size was nevertheless the best predictor of H/L ratio. A number of studies have shown H/L ratio to be sensitive to increased plasma corticosterone (Morici *et al.* 1997; Post *et al.* 2003), levels of which are elevated in stressed individuals, thus providing a reliable physiological indicator of stress in both domestic (Gross & Siegel 1983; Maxwell 1993) and wild (Vleck

et al. 2000; Nephew & Romero 2003; Tompkins *et al.* 2006) bird populations. The fact that I found populations from smaller islands to have higher H/L ratios provides evidence that such populations are exhibiting higher levels of stress than populations on larger islands. Small island sizes also typically lead to dense bird populations which can cause elevated stress levels as is evident in this study, with saddleback populations of higher density exhibiting higher H/L ratios. A high population density is likely to cause an increase in stress levels through increased competition for food, territories, roosting sights, mates and so forth. Furthermore, high densities may directly facilitate the transmission of parasitic organisms (Armbruster & Reed 2005).

Ongoing (days to weeks or more) high levels of stress have been shown to have a depressive effect on the immune system, depleting lymphoid cells essential for optimal immune function (Wikelski & Cooke 2006), hence increasing the risk of infection by parasites and disease. I did not observe any changes in immune parameters with island size apart from an increase in heterophil numbers. The latter is associated with increased corticosterone levels (and inflammatory diseases) (Morici *et al.* 1997). However, I did find lower levels of lymphocytes, and also higher H/L ratios, to be related to founder number. This suggests that genetic stress (i.e., reduced genetic heterozygosity and/or inbreeding) may be more important in terms of reduced function of the immune system than environmental stress. The loss of specific resistance alleles or MHC diversity is believed to be a key factor in pathogen resistance (Benjamini *et al.* 2000) and small population sizes, which are often an inevitable outcome of small island size, are known to increase a population's inbreeding coefficient (Frankham 1998). Thus, a smaller island size can act to exacerbate the effects of inbreeding, the effects of which further predispose populations to

environmental stress and introduction of disease (Frankham 1995). If populations on small islands were also founded by small numbers, not only do they have a reduced level of immune function, they also have higher stress levels both of which may act in synergy to increase the risk of infection by parasitic organisms.

One key finding of my study was the relationship between founder number and feather mite load: feather mite loads were higher in populations founded by fewer individuals. Feather mites are highly specialised, obligatory permanent ectoparasites of a bird's plumage (Dabert & Mironov 1999), feeding on the oil that impregnates the feathers as well as on other material such as bacteria and fungal spores trapped in the oil (Jovani *et al.* 2001). It has been suggested they exert little direct harm on their host and are independent of host immunological constraints (Blanco & Tella 2001; Jovani *et al.* 2001) even though one would expect hosting feather mites would be costly in terms of energy and resources if high feather mite loads were associated with a need for increased production of oils which are essential for the maintenance of feather condition. However, it is thought that the production of oil is probably not regulated by mite numbers as such, instead the hosts hormonal levels are believed to be responsible for this (Blanco & Tella 2001 and references therein). Furthermore, there is evidence to suggest that feather mites may not be parasites at all but rather commensal browsers that are possibly even beneficial to the host (Harper 1999; Blanco & Tella 2001). For example, when the host is unable to preen efficiently either due to ill state of health or beak abnormalities, old oil and detritus may accumulate on the feathers in which pathogenic organisms can proliferate to the detriment of the hosts' health. Subsequently feather mites could increase in numbers and remove the old oil directly affecting pathogens by consuming them together with the feather oil

(Blanco & Tella 2001). Thus, while the high feather mite loads observed on saddleback from more bottlenecked populations may not be directly due to a lower immunocompetence as a result of reduced genetic heterozygosity, they may nevertheless provide an indicator of birds in poor physiological or morphological condition (Blanco & Tella 2001). Therefore, the relationship between feather mite load and founder number may be an indirect result of the effect of low founder number on some other factor which normally acts to prevent such high loads.

Since preening is the first line of defence in the control of ectoparasites and dispersal of oil through the feathers and the precision of preening is in part a function of bill morphology (Clayton 1991), a high mite load could indicate bill abnormalities. Clayton *et al.* (1999) found that feral pigeons with impaired preening due to slight bill deformities had higher parasite loads than individuals with normal bills. Further research showed that bill maxillary overhang plays an important role in ectoparasite control with species that had longer overhangs having fewer ectoparasites (Clayton *et al.* 2005). While I did look at bill asymmetry, I did not directly assess the morphology of saddleback bills and therefore cannot be certain that this is the main cause of the high feather mite loads. However, in my study of saddlebacks I did observe a few individuals with poor horizontal alignment of the upper and lower mandibles and bill tip deformities or damage (e.g., upper and lower mandibles not meeting towards the tip). I did not quantify these abnormalities but such observations provide anecdotal evidence that suggests bill abnormalities are present and further investigation of the relationship between bill morphology, inbreeding and parasite loads is warranted.

Another striking result of this study was the distinct disparity in host response to the three different parasites I observed, in particular an increase in tarsus asymmetry (FA10a, which factors out measurement error) with increasing hippoboscids and decreasing coccidia loads. A large number of studies have demonstrated elevated asymmetry to be associated with parasitism (Blanco *et al.* 2001; Brown & Brown 2002; Perez-Tris *et al.* 2002; Bize *et al.* 2004), the popular suggestion being that resources that would otherwise be allocated towards developmental stability are diverted towards mounting an effective immune response against parasites (Bize *et al.* 2004). However, while increased parasite loads may be a direct reflection of parasite-immune tradeoffs, why would the increase in one parasite and the decrease of another parasite both increase asymmetry? The answer may lie in how costly the parasite is to the host and how many resources must be diverted to immune function as a result. Coccidia (Protozoa, Apicomplexa, *Isospora* sp) are intracellular protozoic parasites that infect the host via a faecal-oral route (i.e., faecal contamination). Large infestations of the parasites in the host can cause a disease called coccidiosis and result in significant damage of the intestinal mucosa and hence a decline of nutritional status and in some cases death (Todd & Hammond 1971; Allen & Fetterer 2002). Thus, the parasite is clearly costly to its host in terms of fitness and survival and it is advantageous to pay the cost of resistance to coccidia.

In contrast, hippoboscids are obligate blood-feeding external parasites and small numbers of these flies (1-4 flies) on the host is thought to be normal for most species of birds on which the parasite has been observed (Hutson 1984; Tompkins *et al.* 1996). The fly is known to be the vector of a number of haematozoa, namely *Haemoproteus* and *Trypanosoma* (Hutson 1984). Very little research has been conducted with regards to host

immune response to hippoboscids flies, but it is assumed that the host is largely able to control hippoboscids loads through behavioural immune mechanisms such as preening and sunning and that it is unlikely that the individual would invest a large amount of energy in mounting an immune response when the cost of doing so is probably far greater than having a small number of flies present. However, as the number of hippoboscids flies increase, it would be reasonable to presume that the impact on the host also increases through blood loss, increased risk of haematozoan infection, lower reproductive success (Tompkins *et al.* 1996) and increased time spent grooming. Thus at this point, there is a significant fitness cost involved and we can expect to see a greater investment of resources in immune function (Møller *et al.* 2003).

The functioning of the immune system is considered to be costly (Lochmiller *et al.* 1993; Norris & Evans 2000) and Møller (2006) hypothesised that the cost of immunity to parasites is paid in term of increased asymmetry. Several studies have shown a trade-off between immune function and growth (Fair *et al.* 1999; Soler *et al.* 2003), providing evidence that mounting an immune response imposes a cost in terms of impaired growth and development. Fair *et al.* (1999) suggest immune responses may interfere with growth and development in highly specific ways, possibly involving disruptions to hormonal and other controls over growth. This is not an unlikely scenario since a number of hormones that regulate growth and metabolism also control lymphocyte-mediated immunity. Growth hormone, prolactin and thyroid hormone all have a general stimulatory effect on lymphocytes. Growth hormone in particular acts within the immune system to positively stimulate lymphocyte mediated responses (Apanius 1998). The diversion of growth hormone and other energy sources away from developmental homeostasis due to increased

immune function could in turn result in increased developmental instability. Therefore, the greater the investment in immunity the greater the level of asymmetry we would expect. This may explain the differences in the relationships I observed between tarsus asymmetry and coccidia and hippoboscids loads.

At first glance it would seem surprising that I only found evidence of a relationship between high parasite loads and increased fluctuating asymmetry in tarsus and not any other traits measured, in particular wattles. According to the FA-sexual selection hypothesis, which is based around the assumption that only high quality individuals should be able to afford to produce sexual traits that are both large and symmetrical (Polak & Starmer 2005), fluctuating asymmetry should be greater in sexually selected characters, such as wattles, that are not critical for immediate survival compared to condition-based traits that may be more strongly canalised (Hoffmann & Parsons 1991; Polak & Starmer 2005). I found no evidence to support this but the lack of such a finding could be explained by the point in the continuum of development at which the individual was exposed to a particular stressor. Since wattles in saddlebacks, and sexual traits in most birds in general, are not developed until they reach sexual maturity, a stressor, such as infection by coccidial parasites, afflicting an individual as a nestling, is less likely to affect wattle symmetry since wattle development occurs much later in the individual's life. Instead, disruption in development will occur with traits developing at the time of the stress (such as tarsi) which are fully developed prior to fledging. Accordingly, fluctuating asymmetry in different traits may represent stress at different stages in development from early ontogeny to sexual maturity. Consequently, studies only observing one trait may be biased by the fact that they are only looking at the effect of stress on one particular part of development, rather than the

entire developmental process. For this reason, the lack of asymmetry in any one particular trait does not necessarily equate to no stress, it could simply be that the potential stressor was not acting upon the individual at the time of development of the trait that was measured.

While I did find a relationship between tarsus asymmetry and founder number, this difference could not be distinguished from measurement error and thus I cannot confidently base any conclusions on such findings. The fact that I found no evidence for a relationship between founder number and FA in any of the traits I measured is consistent with a number of other studies (e.g. Kieser & Groeneveld 1991; Fowler & Whitlock 1994; Gilligan *et al.* 2000;). Gilligan *et al.* (2000) suggest a threshold relationship could exist between FA and genetic diversity, such that developmental instability does not become apparent until a certain level of inbreeding coefficient (F) is reached or alternatively FA may not increase over a certain F . This could be possible in saddleback since intensive research on the North Island saddleback has found low overall genetic diversity for the entire subspecies (Lambert *et al.* 2005) and the same is thought to be likely for the South Island saddleback (Ian Jamieson, pers comm.). Thus all populations of saddleback may be either above the required F threshold or are all below it and hence no developmental instability-founder number relationship is evident.

In contrast to my results on FA, I did find some evidence to suggest there is a threshold founder number for parasite loads and immune function, below which the detrimental effects of the loss of genetic diversity are relatively high. Based on my data for feather mite loads and blood parameters (figures 4.2 and 4.4), it appears that this threshold sits around a bottleneck size of 90 to 110 individuals, as below this range there is a sharp

increase in feather mite loads and H/L ratio and also a sharp decrease in lymphocytes. However, the cluster of the points evident below this bottleneck size tends to suggest that the effects do not worsen below this point. In other words, the negative effects of loss of genetic diversity appear unlikely to differ significantly whether you use 10 individuals or 50 individuals but are likely to improve significantly if greater than 90 founder individuals are used. This effect may simply be due to both measures obtaining their most extreme values possible (i.e., feather mite loads cannot physically increase any further due to intrinsic limits on the life history of the parasite). Clearly, conservation managers should strive to found future populations of this species (and perhaps supplement current populations of saddlebacks founded with few founders) to a size that exceeds this apparent threshold if the deleterious effects of bottlenecks on immune function are to be minimised.

An alternative explanation for the lack of any relationship between founder number and FA could simply have been due to differences in environmental conditions between the islands and/or low statistical power due to small sample sizes. It is becoming increasingly apparent that large sample sizes are required for the accurate estimation of fluctuating asymmetry and that fluctuating asymmetry experiments are fraught with error and inconsistencies (Gilligan *et al.* 2000). This raises the question as to the value of using fluctuating asymmetry in detecting stress in endangered populations which are, by their very nature, scarce. Physiological methods such as H/L ratio, immune parameters, and corticosteroid levels, appear to be more reliable at identifying present ongoing stress, particularly in endangered species whereas fluctuating asymmetry may be more relevant in studies assessing resource allocation such as in the trade-off between immune function, growth and parasite infection.

My results confirm that small island size and founder number are key factors contributing elevated stress levels in endangered populations increasing susceptibility to parasitic outbreaks. This is of particular concern considering the escalating amount of habitat fragmentation as the level of human activity rises together with increasing emergence and dispersal of disease. Loss of genetic diversity through severe population bottleneck events and inbreeding may mean many species will not be able to adapt to these changes and will ultimately become extinct as a result. There is growing evidence to suggest that loss of genetic diversity is a large contributing factor in increased disease outbreaks in endangered populations and lower tolerance to environmental extremes (e.g. Keller *et al.* 1994; O'Brien & O'Brien *et al.* 1985; O'Brien & Evermann 1988; Thorne & Williams 1988). My study confirms that conservation managers must find ways of detecting stressed populations and alleviating the stress before the survival of the population or organism is irreversibly compromised.

4.7 References

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Chapter 5

**Population bottlenecks and the expression of a morphological
secondary sexual trait in male and female saddlebacks
(*Philesturnus carunculatus*)**

5.1 Abstract

It has only recently been suggested that individual heterozygosity may be the underlying causative factor of many phenotypic correlations supporting theories of parasite-mediated sexual selection. For example, individuals with higher immunocompetence and larger ornaments may in turn have higher levels of genetic heterozygosity. This addition to the good genes theories of mate choice nevertheless remains focused on secondary sexual ornaments indicating the genetic quality of males only. However, females are likewise negatively affected by inbreeding and, in species with ornamented females, such ornaments may also signal her genetic quality. The present study investigates the relationship between wattle size in both male and female saddlebacks with bottleneck size, parasite load and immune parameters. My results show that wattle size is apparently unaffected by founder number; however, I did find a significant relationship between feather mite loads and wattle size in both males and females, with individuals exhibiting larger wattles having fewer parasites. This may indicate a trade-off, for example between time spent preening and time spent foraging for foods, particularly those containing the carotenoids needed for large wattles. Finally, I found the males and females with larger wattles had higher lymphocyte counts and lower H/L ratios suggesting higher immune function and lower levels of stress than in individuals with smaller wattles. This finding also suggests that wattle size signals female quality in terms of immune function. I discuss the role of the female in the early development of the immune system of offspring and suggest she may play a greater role in offspring fitness and early immunocompetence than has been appreciated in conventional views of sexual selection.

5.2 Introduction

Since Darwin (1871) first introduced the idea that female preference for ornamented males may act as a strong selective force there have been a number of theories which attempt to explain the elaborate ornamentation of males. Most studies have focused on the “good genes” theory of sexual selection, whereby females selecting males possessing elaborate ornaments are expected to benefit by mating with males that are resistant to current parasites (parasite-mediated sexual selection hypothesis: Hamilton & Zuk 1982) and have enough resources for both elaborate ornaments and a high degree of immune function (immunocompetence handicap hypothesis: Folstad & Karter 1992; Wedekind & Folstad 1994). Thus, by choosing a male with the largest or brightest trait, the female can ensure her offspring inherit high quality genes and will subsequently have a high level of fitness. Although genetically-based theories of sexual selection propose that elaborate sexual ornaments of males are indicative of some aspect of his genetic quality, few studies have investigated the effect of inbreeding, and specifically the loss of genetic diversity, on the expression of secondary sexual ornaments (Landry *et al.* 2001; van Oosterhout *et al.* 2003; Reid *et al.* 2005; Mariette *et al.* 2006).

Inbreeding, the mating between individuals who are more closely related than by chance alone, can manifest as inbreeding depression, the resultant loss of fitness leading to reduction in lifetime reproductive success (Keller 1998; Slate 2000), low survivorship, reduced resistance to disease (Spielman *et al.* 2004), physical deformity (e.g. Seymour *et al.* 2001; Polak 2003) and increased extinction risk (Frankham 1995; Brook *et al.* 2002). There has been a long-standing interest in the consequences of inbreeding in domestic and captive animals (Charlesworth & Charlesworth 1987); however, the fitness consequences

of inbreeding in wild populations have only recently been brought to attention (Frankham *et al.* 2002; Keller & Waller 2002; Reed & Frankham 2003). Increasingly, studies indicate that inbreeding is more common in wild populations than previously thought and that inbreeding can have a wide array of detrimental effects in these populations (e.g. Thorne & Williams 1988; Saccheri *et al.* 1998; Mackintosh & Briskie 2005 but see Keller & Waller 2002). Since inbreeding is associated with loss of fitness, the very basis of ‘good genes’ theories of sexual selection, it would seem intuitively logical that inbreeding could have an effect on the expression of sexual ornaments.

Although the detrimental effects of loss of genetic diversity are now clear, it is has only recently been suggested that sexual ornaments may signal the level of heterozygosity in an individual. Brown’s (1997) ‘good genes as heterozygosity’ theory predicts that male ornament expression, symmetry of ornaments and male mating success will be positively correlated with the degree of heterozygosity. The few studies that have looked at sexually selected traits in the context of inbreeding (and thus an assumed loss of heterozygosity) have produced mixed conclusions, with many studies finding a relationship between inbreeding and behavioural traits (e.g. song, courtship displays: Tregenza & Wedell 2002; Foerster *et al.* 2003; Reid *et al.* 2005; Mariette *et al.* 2006) but not morphological traits (e.g. colouration, Mariette *et al.* 2006). Reid *et al.* (2005) developed Brown’s (1997) theory further suggesting that because heterozygosity is known to influence immunity, a phenotypic correlation between ornamentation and immunity could be explained by the level of heterozygosity as opposed to any parasite-mediated effect as has been the traditional interpretation of many such correlations (e.g. Hamilton & Zuk 1982; Møller 1990). Using a pedigreed population of song sparrows (*Melospiza melodia*), Reid *et al.*

(2005) provide evidence in support of their theory, showing both male song repertoire size and cell-mediated immune response declined with the coefficient of inbreeding.

In this chapter I investigated the relationship between wattle size and bottleneck size, parasite load and a number of immune parameters in male and female saddlebacks (*Philesturnus carunculatus*). I compared 12 populations of saddlebacks differing in founder number, to determine if populations that have been through more severe bottlenecks have smaller wattles, and similarly if those with smaller wattles have higher parasite loads and/or lower immune function. I assumed that individuals in populations that had been through more severe bottlenecks were more likely to have reduced heterozygosity, a finding that has some support (Leberg 1991; Taylor *et al.* 1994; Houlden *et al.* 1996). Wattles on many bird species are one example of a secondary sexually selected trait, and are thought to influence female mate choice. However, in the saddleback, both male and female develop wattles indicating that there may be some degree of mutual mate choice or male choosiness in this species, as has been found in other species of birds in which both sexes display sexually-selected ornaments (Jones & Hunter 1993; Kraak & Bakker 1998; Roulin *et al.* 2001a).

Traditionally, studies of sexual selection by mate choice have focused solely on the female being the choosy sex, and it is only recently that studies are beginning to indicate that males may also be choosy and that female quality may be equally important in some species (e.g. Amundsen *et al.* 1997; Amundsen 2000; Hanssen *et al.* 2006). Hence by looking at both male and female wattles in the saddleback I have an ideal opportunity to determine if wattles are indicative of female quality or if they are simply genetic correlates of male traits, and serving no purpose as has been historically suggested (Lande & Arnold

1985). Thus, I expand on the hypothesis of Reid *et al.* (2005) to include females and determine the role of reduced genetic variation through population bottlenecks in a morphological secondary sexual trait expressed by both males and females.

5.3 Methods

5.3.1 Study populations

The saddleback is one of three species in the unique wattlebird family Callaeidae which includes the kokako (*Callaeas cinerea*) and the extinct huia (*Heteralocha acutirostris*). This medium-sized (70-90 g) cavity nesting passerine is striking for its chestnut-coloured saddle and rump, which is distinctive from the otherwise glossy black plumage and fleshy orange-red wattles at the base of the bill (Figure 5.1). The smaller overall size of the female (70 g, versus 80 g in males), including the wattles, is the only obvious dimorphism between the otherwise identical sexes. There are two distinct geographical subspecies of the saddleback: *P. c. carunculatus* in the North Island and *P. c. rufusater* in the South and Stewart islands. Both are identical in colouration in the adult stage, except for a thin gold band at the upper edge of the saddle in the North Island saddleback. The adults and juveniles are easily distinguished in this species based on plumage differences, the juvenile of the South Island saddleback being a drab rust colour (they are commonly referred to as jackbirds) and the juvenile of the North Island saddleback lacking the thin gold band at the upper edge of the saddle.

Both subspecies were widespread and plentiful when Europeans first arrived but were reduced to two single offshore island populations following the introduction of rats and mustelids. The North Island saddleback declined to only 500 individuals, surviving on Hen Island, and the South Island saddleback to only 35 survivors on Big South Cape Island. Following a series of translocations, both subspecies now thrive on over 20 predator free offshore islands around New Zealand (Hooson & Jamieson 2003). The present study includes North Island saddleback populations on Hen (the source population, 484 ha),

Cuvier (170 ha), Lady Alice (155 ha), Tiritiri Matangi (197 ha), Mokoia (135 ha) and Kapiti (1965 ha) islands, and South Island saddleback populations on Big (23 ha), Kaimohu (11 ha), Putauhinu (141 ha), Breaksea (170 ha), Motuara (59 ha) and Ulva (270 ha) islands (Table 5.1). The 12 saddleback populations in my study differ in their founder number (ranging from 15 to 500 individuals), thus providing an ideal opportunity to determine if those populations that have gone through more severe bottlenecks (fewer founders) have smaller wattles as a consequence of reduced genetic diversity. I also included island size and the density of saddleback on each of the islands, as both may be strong environmental confounding factors that could account for any differences seen in wattle size. Population density was obtained from published data (Lovegrove 1996; Hooson & Jamieson 2003) and personal communication with the Department of Conservation.



Fig. 5.1 Adult saddlebacks. Top: Male North Island saddleback, showing a close up view of the head with the orange fleshy wattles at the base of the bill. Bottom: Female South Island saddleback, showing the chestnut coloured saddle and rump and relatively smaller wattles.

5.3.2 Wattle size

Between October 2002 and August 2006 I caught 289 adult saddlebacks using mist-nets, and banded each individual with a unique colour code to allow for individual identification. Two independent measurements of the length of the left and right wattles were made to the nearest 0.01 mm with slide callipers which were reset to zero between measurements. The sequence of measurement was always right-left-right-left. The measurements were then averaged to give the average wattle length for the individual. All measurements included in this study were carried out by myself to avoid any errors associated with different people. Any outliers (due to bad measurement or aberrant individuals) were identified using Grubb's test (Sokal & Rohlf 1995) and excluded from the final results (3 out of 289 birds in total).

5.3.3 Parasite loads

An estimate of feather mite density was obtained for each individual by examining the primary feathers of the left wing. Feather mite density was given a category score from 0 – 5 where: 0 = no feather-mites; 1 = 0-10; 2 = 10-100; 3 = 100-1000; 4 = 1000-10000 and 5 = 10000+ feather-mites. The number of hippoboscids flies (*Ornithomya* spp. and *Ornithoica* spp.) seen on or flying off the bird were also counted. Faecal samples were collected for analysis for gastrointestinal nematodes and the protozoan parasite coccidia. Standard faecal flotation techniques carried out at a commercial laboratory (New Zealand Veterinary Pathology Ltd, Hamilton) were used to obtain parasite oocyst counts providing an estimate of coccidia and nematode burdens.

5.3.4 Immune parameters

A drop of blood was drawn from the right wing of each individual via brachial venipuncture to measure leukocyte parameters, which can provide an estimate of the immune condition of a bird (Woerpel & Rosskopf 1984; Fudge 2000). Blood was smeared onto a glass slide, fixed in methanol and stained using a modified May-Grünwald Giemsa staining method (Lucas & Jamroz 1961). Blood smears were then viewed under a light microscope and the following measurements taken: (1) total leukocyte number (leukocyte count), which can give an indication of the overall health of the individual at the time of sampling (Woerpel & Rosskopf 1984) and is calculated by counting all leukocytes in ten consecutive 400x fields of view, in an area where the cells are in a monolayer showing the most uniform distribution of erythrocytes (normally close to the feathered edge). These counts are then averaged to give an estimate for each individual (Walberg 2001); and (2) a leukocyte differential, which may be indicative of disease processes and stress responses (Woerpel & Rosskopf 1984), was obtained by examining each blood smear under oil immersion (1000x) and determining the relative frequency of the five different types of leukocyte (lymphocytes, heterophils, basophils, eosinophils and monocytes) for a total of 100 leukocytes. The heterophil/lymphocyte ratio (H/L), which has been used as an index of physiological stress in both poultry (Gross & Siegel 1983; Maxwell 1993) and wild birds (Tompkins *et al.* 2006), was calculated by dividing the number of heterophils by the number of lymphocytes. Finally, the blood smear was scanned for three minutes using a cross-sectional (up-across-down-across-up, etc.) scanning method to detect any blood borne parasites.

5.3.5 Statistical analyses

I performed all statistical analyses in Statistica 6 (StatsSoft, Inc.). Data transformations were applied to variables that did not conform to normality. I used general linear models to determine if there were any significant differences between sexes and islands in wattle size. I used general regression models to determine if founder number and the number of population bottlenecks were related to variation in wattle size for either sex including island size and saddleback population density in the model to control for possible environmental confounding factors. I used Pearson correlations to determine if variation in wattle size was related to any of the parasite or immune parameter measure. Because wattle size correlated significantly with body mass overall ($r = 0.36$, $N = 286$, $P < 0.0001$), and for females ($r = 0.19$, $N = 152$, $P < 0.05$) but not for males ($r = -0.13$, $N = 134$, $P = 0.13$), I also carried out each analysis controlling for body mass as it could confound any relationship between wattle size and the predictor variables. I did this by regressing wattle size against body mass for each sex and used the residuals of both these relationships as the dependent variable in the analyses which controlled for weight.

5.4 Results

5.4.1 Wattle size and founder number

There was a significant difference between sexes in wattle length ($F_{1,284} = 84.51$, $P < 0.0001$); males on average have larger wattles than females. However, when body mass was controlled for there was no significant difference in wattle size between the two sexes (ANOVA, $P > 0.05$). There was also a significant difference between island and wattle length and this held true for both sexes (male: $F_{11,122} = 12.96$, $P < 0.0001$; female: $F_{11,140} = 7.02$, $P < 0.0001$). I found no significant relationship between mean wattle length and either founder number, the number of bottleneck events, island size, or the number of birds per hectare for either sex (male: $F_{4,7} = 0.45$, $P = 0.77$; female: $F_{4,7} = 0.49$, $P = 0.74$), and this held true when body size was controlled for (male: $F_{4,7} = 1.65$, $P = 0.26$; female: $F_{4,7} = 0.59$, $P = 0.68$). Thus, wattle size did not appear to be related to the size of the bottleneck or the number of bottlenecks each population had passed through.

5.4.2 Wattle size and parasite load

Not all islands were found to possess hippoboscids and/or coccidia, however saddleback from all 12 populations harboured feather mites (Table 5.1). There was no significant relationship between female or male wattle size and either coccidia or hippoboscid loads and this relationship held true whether or not body weight was controlled (Pearson correlation coefficients, all $P > 0.05$). A significant correlation did exist between male wattle size and feather mite loads ($r = -0.65$, $N = 12$, $P < 0.05$; Fig. 5.2a); males with larger wattles had fewer feather mites. However this relationship, whilst still present, was non-significant when body mass was controlled ($r = -0.52$, $N = 12$, $P = 0.09$; Fig. 5.2b).

Similarly there was a significant correlation between female wattle size and feather mite loads ($r = -0.61$, $N = 12$, $P < 0.05$: Fig. 5.2c), but unlike males the relationship remained significant when female weight was controlled ($r = -0.68$, $N = 12$, $P < 0.05$: Fig. 5.2d). No blood parasites were seen from the blood smears of any saddleback from any population.

5.4.3 Wattle size and immune parameters

Male wattle size correlated significantly with H/L ratio ($r = -0.66$, $N = 12$, $P < 0.05$) and lymphocyte ($r = 0.68$, $N = 12$, $P < 0.05$) and eosinophil ($r = -0.66$, $N = 12$, $P < 0.05$) counts, and this same relationship held when male weight was controlled (H/L: $r = -0.65$, $N = 12$, $P < 0.05$; lymphocytes: $r = 0.65$, $N = 12$, $P < 0.05$; eosinophils: $r = -0.60$, $N = 12$, $P < 0.05$: Fig. 5.3a-c). Males with larger wattles had lower H/L ratios and eosinophil counts and higher lymphocyte counts. The pattern was only slightly different in females, with wattle size correlating significantly with H/L ratio ($r = -0.62$, $N = 12$, $P < 0.05$) and lymphocyte ($r = 0.60$, $N = 12$, $P < 0.05$) and heterophil ($r = -0.58$, $N = 12$, $P < 0.05$) counts. However, when body weight was controlled for only H/L ratio ($r = -0.57$, $N = 12$, $P = 0.05$: Fig. 5.3d) and lymphocyte count ($r = 0.69$, $N = 12$, $P < 0.05$: Fig. 5.3e) correlated significantly with female wattle size. Nevertheless, in both cases, females with larger wattles had higher lymphocyte counts and lower H/L ratios.

Table 5.1 Island size, population founding number and mean wattle size and parasite loads of saddlebacks.

population	founder #	island size (ha)	wattles					parasite loads		
			mean length (mm)					feather mite	hippoboscid	Isospora sp
			sexes combined	N	male	N	female			
Hen	500	484	10.56	15	11.02	12	8.75	0.60	0.07	1573
Cuvier	29	170	8.62	29	9.59	11	8.03	0.21	0.21	1267
Lady Alice	21	155	9.28	25	10.11	16	7.92	3.04	0.00	7242
Tiritiri Matangi	29	197	8.19	49	9.33	20	7.41	2.10	0.68	15451
Mokoia	36	135	9.17	21	9.57	11	8.72	1.61	0.28	0
Kapiti	122	1965	8.57	24	9.09	11	8.13	0.92	0.09	9340
Big	21	23	6.66	28	7.21	7	6.47	4.22	0.00	0
Kaimohu	15	11	6.88	12	7.65	5	6.33	4.58	0.00	416
Putauhinu	65	141	7.57	12	8.56	6	6.58	4.08	0.00	18809
Breaksea	59	170	5.68	14	5.97	9	5.16	3.23	0.00	18675
Ulva	30	270	7.97	12	8.49	3	7.79	3.90	0.00	*
Motuara	26	59	8.85	45	9.57	23	8.09	3.77	1.53	1826

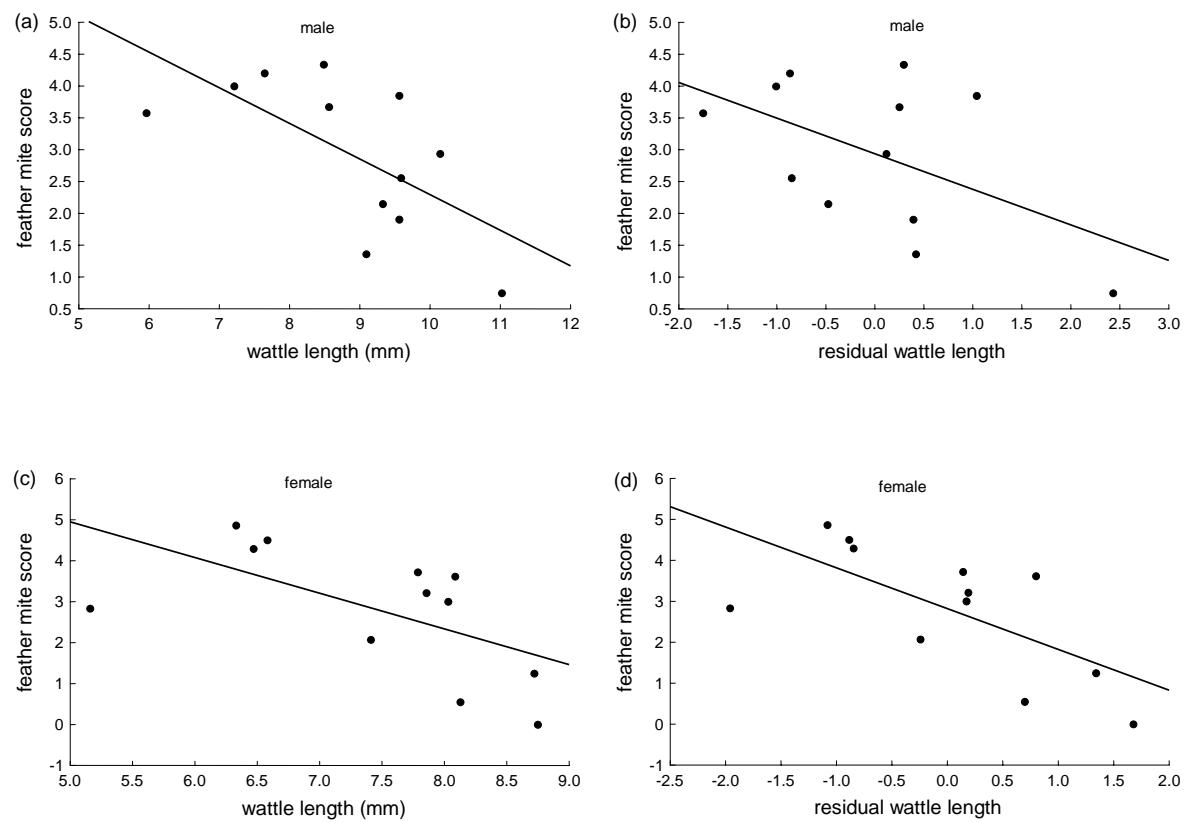


Figure 5.2 Relationship between wattle length and feather mite score in males (a) without correcting for body mass and (b) correcting for body mass and between wattle length and feather mite score in females (c) body mass not corrected and (d) body mass corrected.

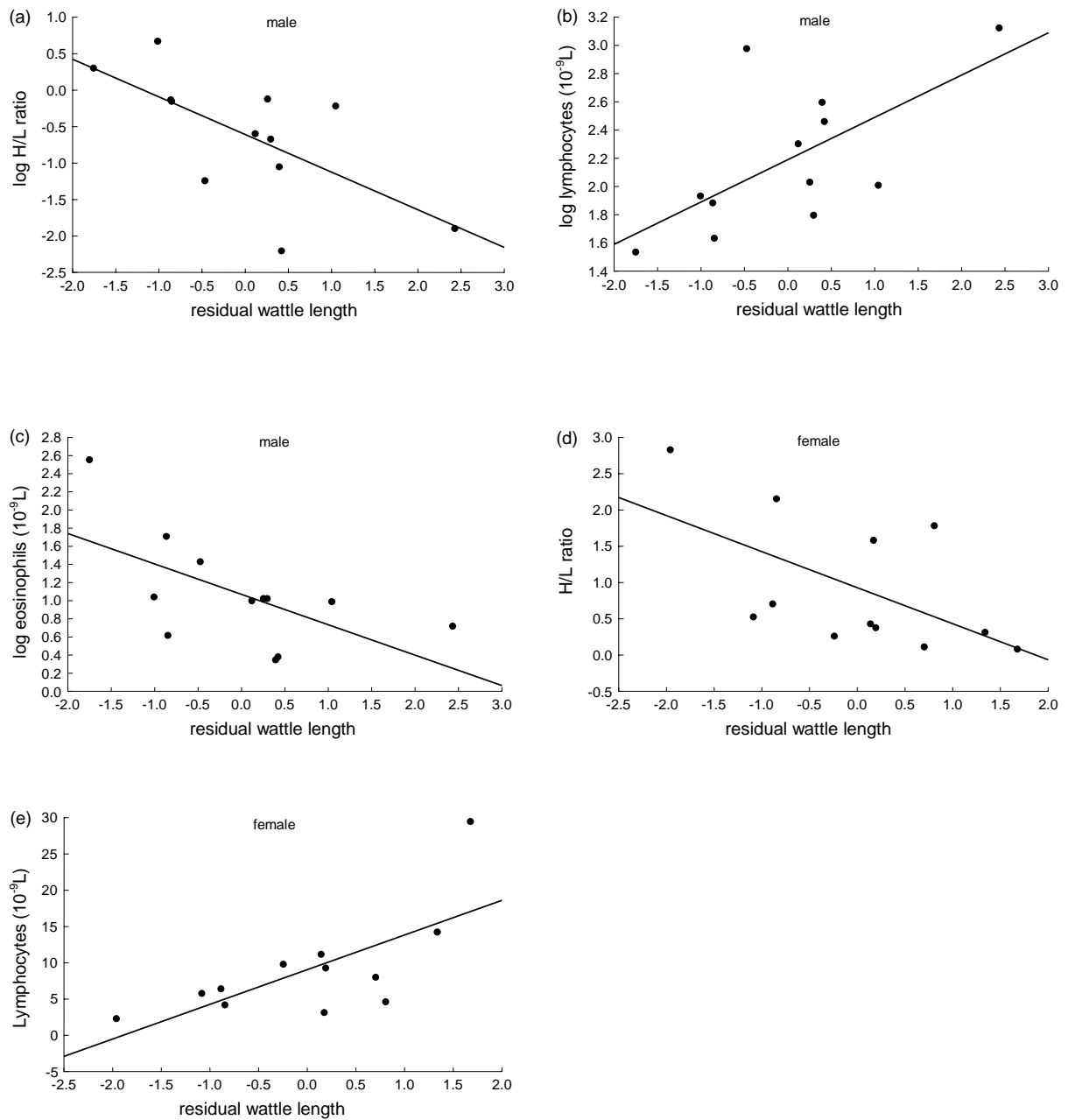


Figure 5.3 Relationship between wattle size and (a) H/L ratio, (b) lymphocytes and (c) eosinophils in males after controlling for body mass and between wattle size and (d) H/L and (e) lymphocytes in females after controlling for body mass.

5.5 Discussion

When I examined 12 populations of saddleback I found that H/L ratio was lower and lymphocyte count was higher for both males and females with larger wattles and this relationship held whether body weight was corrected for or not. This suggests that individuals with larger wattles have lower levels of stress and higher levels of immune function. Additionally, I found that males with larger wattles had lower eosinophil counts and this again held true whether or not body weight was corrected. In contrast, females with larger wattles had lower heterophil counts however this relationship did not exist when body weight was factored into the equation. Individuals with larger wattles in both sexes exhibited lower feather mite counts although when body weight was controlled the relationship only remained for females. I found no relationship between wattle size and either coccidia or hippoboscids loads or any relationship between wattle size and founder number or the number of bottlenecks the population had been through for either males or females and this held true whether or not the data was corrected for body mass.

The simplest explanation for the lack of any relationship between founder number and the number of bottlenecks and wattle size in saddlebacks is that there is no effect of population bottlenecks on wattle length, at least for the element of the wattle I measured. My findings are consistent with a recent study carried out by Mariette *et al.* (2006), who found no significant influence of inbreeding on colour pattern variation in guppies although they did find a significant effect of inbreeding on courtship behaviour. Several other studies have also found significant effects of inbreeding on behavioural sexual traits (Meffert 1995; Aspi 2000; van Oosterhout *et al.* 2003; Reid *et al.* 2005), thus the lack of any effect of bottleneck size on wattle size in saddlebacks could be due to the type of

secondary sexual trait examined (morphological as opposed to behavioural). Different traits undergo different developmental pathways and subsequently may entail dissimilar constraints and costs. For example, red/orange pigmented morphological traits, such as the wattles on saddlebacks, may require carotenoids which cannot be synthesised by animals and thus must be obtained from foods (Badyaev & Hill 2000). Ornaments which utilise carotenoids are considered costly to produce since carotenoids are believed to be scarce in nature and difficult to metabolise (Olson & Owens 1998). Due to the close link between carotenoids and diet, such ornaments may be more influenced by environmental variation, in particular diet, than levels of genetic heterozygosity and may also be less developmentally constrained compared to melanin-based (black, brown, etc.) or behavioural traits (courtship, song, etc.) (Badyaev & Hill 2000). In contrast, these latter traits are thought to be under more rigid genetic control and not as easily affected by environmental variation such as diet (Badyaev & Hill 2000). This suggests that behavioural/melanin-based secondary sexual traits may be more reliable in detecting any bottleneck effects whereas those based on carotenoid-pigments are more reliable condition indicators. Since I did not consider any other secondary sexual traits such as plumage colouration and song, I cannot rule out any effects of bottleneck size or number on sexual ornamentation in saddlebacks. Thus it is worthwhile for future studies to include multiple sexual traits since different traits may indicate different underlying qualities of the individual whether they are based on condition or 'good genes' (Lindstrom & Lundstrom 2000).

Alternatively the lack of any relationship between bottleneck size or number and wattle length could be due to the fact that bottleneck size and the number of bottlenecks

may be unreliable measures of genetic heterozygosity in the individuals I measured. My research was based on the assumption that more severe bottlenecks reduce heterozygosity in all individuals in all populations. However it has been suggested that this is not always the case (Ardern *et al.* 1997). Nevertheless, this seems very unlikely based on the evidence in Chapters 3 and 4 showing significant effects of bottleneck size on a number of immune parameters (namely H/L ratio and lymphocyte counts) and feather mite loads and strong experimental evidence in Chapter 2 confirming the negative effect of small bottleneck size on the cell-mediated immune response.

I examined a variety of internal and external parasites in each of the saddleback populations and found no significant relationship between wattle size and loads of coccidia or hippoboscids flies. I did find a significant relationship between wattle size and feather mite load: both males and females with fewer feather mites had larger wattles. However, when body weight was taken into account the latter relationship was no longer significant for males suggesting that feather mite load may be solely due to the fact that bigger birds, which tend to have bigger wattles, have a larger surface area in which to host more feather mites. Nevertheless, unlike females, male wattle size did not correlate with body mass and would suggest this may not necessarily be the case. The fact that only feather mites related to wattle size is surprising since both hippoboscids flies and coccidial parasites are known to have direct impacts on their hosts, the former associated with blood loss and blood parasite transmission (Hutson 1984; Tompkins *et al.* 1996) and the latter with damage of the intestinal mucosa and in severe cases death of its host (Allen & Fetterer 2002; Todd & Hammond 1971). Both parasites compete with host resources, particularly coccidia, which

has been shown to have an effect on carotenoid metabolism and have a negative impact on the expression of secondary sexual traits (Horak *et al.* 2004).

In contrast, feather mites are believed to exert little direct harm on their host and to be largely independent of host immunological constraints (Jovani *et al.* 2001). Instead, these highly specialised, obligatory permanent ectoparasites of bird's plumage (Dabert & Mironov 1999) are thought to be beneficial to the host due to their feather cleaning actions (Blanco & Tella 2001; Jovani *et al.* 2001). However, the findings of my study and those of others (e.g. Thompson *et al.* 1997; Harper 1999) suggest otherwise. It is logical to presume a high feather mite load would be costly in terms of energy and resources for the birds to produce preen gland oils necessary for the maintenance of feather condition. High feather mite loads would mean more oil is consumed and presumably the bird must produce more to make up for the lost oil and more time may have to be spent preening at the cost of time spent carrying out other activities. However, it is thought that the production of oil is regulated by hormone levels not feather mites as such (Blanco & Tella 2001 and references therein) and there is evidence to suggest that feather mites may not be parasites at all but rather commensal browsers, browsing on old oil and fungus and pathogens trapped in that oil (Blanco & Tella 2001). Clearly more work is needed to determine the exact costs and benefits to birds of harbouring feather mites before their role (if any) in the evolution of sexual signals can be more fully understood.

One well known weakness of correlation studies is that they do not isolate the cause of the effect, therefore while I found feather mite numbers to be significantly related to wattle size it they may not necessarily be the primary cause of wattle size variability. Instead, variation in feather mite loads may be the indirect result of another underlying

factor that I did not measure, which in turn is related to wattle size. If the red colouration of saddleback wattles is derived from carotenoid pigments, then one potential factor could be the trade-off between preening behaviour and foraging behaviour. Because carotenoids are rare in nature a considerable amount of time spent in foraging would be expected, at the cost of time spent preening. Thus poor quality individuals, possibly with poor territories, would be forced to spend more time foraging and less time preening, thereby allowing feather mite loads to build up and possibly also stress levels as is evident for both male and female saddleback from populations with smaller wattles. Furthermore, far larger amounts of carotenoids are needed for the production of carotenoid-based pigmented ornaments than can be obtained in the food (Badyaev & Hill 2000) and hence carotenoids stored in the body may be diverted away from other uses such as immune function, in order to produce larger wattles. It is expected that only individuals in good condition with large carotenoid stores will be able to spend more time preening (hence fewer parasites), and can afford to divert more carotenoids away from the immune system to produce larger, brighter non-functional ornaments.

The core idea of Hamilton and Zuk's (1982) hypothesis is that high quality males sire the healthiest young that are resistant to particular parasites largely due to having inherited those genes from their father. This theory predicts that these males also have the genetic make-up that provides better immune defence against pathogens (Folstad & Karter 1992; Westneat & Birkhead 1998). Ideally to test this theory, the immune function of individuals would be determined using experimental techniques such as the phytohaemagglutinin test and sheep red blood cell (SRBC) hemagglutination assay for antibody-mediated immunity. I was unable to use such experiments in this study due to

permit restrictions; nevertheless, in the absence of disease, comparatively higher sustained leukocyte and lymphocyte counts can be associated with higher levels of immune function (Gustafsson *et al.* 1994; Adamo 2004). The higher lymphocyte counts I observed suggest that male saddlebacks with larger wattles may have higher levels of immunocompetence. That I found lower eosinophil counts, high levels of which are suggested to be linked with high parasite loads (Fudge 2000), together with lower feather mite loads in males with larger wattles further suggests that larger wattles in male saddleback may indicate his superior resistance to parasites.

One striking result of my study was that females with larger wattles also had higher lymphocyte counts and this has significance both in terms of female quality and her role in early immune defence in the offspring. The protective effects of maternal antibodies and maternal influence on neonatal immune development and protection has long been recognised (Grindstaff *et al.* 2003 and references therein). Because B-lymphocytes are responsible for the production of immunoglobulins (Klasing & Leshchinsky 1998), I would expect that higher levels of lymphocytes in an individual indicates higher levels of immunoglobulins. Thus the higher levels of lymphocytes in females with larger wattles may mean that larger wattles signal her ability to produce and transfer a larger quantity and diversity of immunoglobulins to the offspring, ensuring greater protection of the young bird to the array of novel pathogens it encounters upon hatching. This early aid may also allow for greater growth given that more immune input from the mother may mean more resources can be directed towards growth (Grindstaff *et al.* 2003). These are potential key factors in the theory of female quality signalling. Since antibody production increases during egg production, and immunoglobulin production and allocation to the eggs is costly

both in terms of nutrition and energy and presumably involves immune and reproductive tradeoffs in the females (Pihlaja *et al.* 2006), only high quality females will be able to produce sufficient antibodies to transfer to her offspring and at the same time produce costly ornaments. Thus, it makes intuitive sense that a male should choose a female with larger wattles, since it signals to him that she has a higher level of immunocompetence and will be able to allocate a larger and more diverse range of immunoglobulins so their offspring will hatch better equipped to face the diverse array of pathogens, possibly have higher growth rates and thus have increased survival prospects.

Few studies have actually found a relationship between male secondary sexual trait expression and the ability to resist parasites (Møller 1990), and although some studies have found a relationship between level of immune function and sexual ornament expression (e.g. Duffy & Ball 2002), few have actually attempted to link male immunocompetence directly to offspring immune function and those that have, have found no association between male and offspring immunocompetence (Kleven *et al.* 2006; Kurtz & Sauer 1999). It would appear good gene theories incorporating immunocompetence have been based on the assumption the offspring inherits the majority if not all aspects of its immune function from the father. However, the significant role the mother plays in offspring immune function, and increasing evidence, including my own, suggesting ornaments also signal quality in females (Amundsen *et al.* 1997; Roulin *et al.* 2001b; Hanssen *et al.* 2006), illustrate this may not be the case.

The present study found no evidence to suggest population bottlenecks affect the expression of a sexually selected trait present in both males and females. Nevertheless, my study highlights a gap in theories of sexual selection: the significant role females play in the

immunocompetence of their offspring. Given the increasing number of studies indicating the negative effect of inbreeding on immune function, it is clear there is a need for future studies that investigate the relationship between inbreeding and secondary sexual traits to include both sexes and relate this back to offspring immune quality in order to obtain a clearer understanding of the role each sex has in offspring immune development and functioning and how this is affected by individual heterozygosity. Furthermore, I recommend caution in drawing any conclusions based on findings analysing single traits and/or single groups of parasites since different traits can signal different qualities and different parasites may affect these traits in dissimilar ways.

5.6 References

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Chapter 6

General Discussion

Global-scale anthropogenic changes have greatly disrupted normal host-parasite dynamics. Changes in weather patterns, habitat degradation, and species introductions are among a host of factors that have greatly amplified the role of diseases as regulating factors in the survival of many species, to the point where they have become agents of extinction (Deem *et al.* 2001; Cleaveland *et al.* 2002; Frankham 2005). That inbreeding increases extinction risk is a view that has been widely challenged (e.g. Harcourt 1991; Young 1991; Caro & Laurenson 1994); however, there is now compelling evidence that inbreeding and the loss of genetic diversity increase the risk of extinction, namely because it reduces the ability of a population to cope with a rapidly changing ecosystem, including new diseases and climatic change (Frankham 2003).

Novel pathogens represent one of the most significant challenges to all species, perhaps even more so to those species already in critical decline. There is a growing list of well documented extinctions or near extinctions of threatened populations that have been the result of disease outbreaks: malaria and avian pox in Hawaiian land birds (van Riper *et al.* 1986), canine distemper virus in the black footed ferret *Mustela nigripes* (Thorne & Williams 1988), and rabies in Kenyan African wild dogs, *Lycaon pictus* (Kat *et al.* 1995). Empirical models indicate that a density threshold exists, below which disease cannot persist and therefore is unlikely to be a problem in rare populations or be the cause of their extinction (Lafferty & Gerber 2002). However, these models do not take into account changes in host susceptibility due to inbreeding, stress and immunosuppression.

The relationship between severe population bottlenecks (and thus increased inbreeding and loss of genetic diversity) and the development and functioning of the immune system has, until recently, received very little attention. However, theoretical and

more recently, experimental studies suggest that inbreeding should increase a bottlenecked population's susceptibility to parasites and infectious diseases as reduced genetic variation in inbred individuals could result in loss of diversity of the major histocompatibility complex (MHC) and in less adaptable immune systems (Potts & Wakeland 1990; Paterson *et al.* 1998; Smits *et al.*, 1999; Zekarias *et al.*, 2002). If this is the case, then many endangered species, which are prone to high levels of inbreeding due to severe population bottlenecks, are in danger of disease outbreaks and possible extinction.

The small size of many New Zealand island and relict populations suggests that inbreeding has long been part of the mating systems of New Zealand birds (Craig 1991). The Chatham Island black robin (*P. traverse*) provides a classic example of a population known to be highly inbred following a severe bottleneck event in the 1970s that left only a single effective breeding pair (Flack 1974). It is accepted that a level of inbreeding is unavoidable in endangered species and that exposure to parasites and pathogens is in fact required in order to obtain a level of immunity (Altizer *et al.* 2001). However, the number of founders needed to establish new populations and at the same time maintain or enhance immunocompetence, prevent crowding, and excessive stress and subsequent disease outbreaks, are fundamental questions that need to be answered in order to achieve successful restoration projects and maintain the long-term viability of endangered species.

Using two New Zealand bird species as models, I identified a number of mechanisms by which inbreeding and loss of genetic diversity could increase disease susceptibility in native bird populations. The findings of chapter two, confirmed that severe bottlenecks reduce the immune response of birds. This is in agreement with three previous experimental studies that have investigated the relationship between genetic

heterozygosity and immunocompetence (Hawley *et al.* 2005; Reid *et al.* 2005; Whiteman *et al.* 2006). Whilst I only took into account one aspect of the immune system, the cell-mediated immune response, and thus cannot rule out that resources were directed towards other sectors of the immune system such as the humoral immune response, the fact that both leukocyte and lymphocyte counts were lower in the bottlenecked population also suggests lower overall immune function (Adamo 2004; Gustafsson *et al.* 1994). That I found a lower response for the phytohaemagglutinin skin test for the post breeding season only, when hippoboscids fly loads on the birds were at their peak, supported predictions that because functioning of the immune system is costly and involves trade-offs, upregulation of the immune system should only occur when parasite numbers are most abundant. In addition, evidence suggests resources are directed away from the immune system during breeding in order to support increased reproductive effort (Deerenberg *et al.* 1997; Merino *et al.* 2000; Dubiec & Cichon 2001; Lozano & Lank 2003; Møller *et al.* 2003). Thus, I suggested caution in interpreting findings from studies based around a single season since functioning of the immune system is not seasonally uniform.

Having closely followed the Motuara Island saddleback population as it recovered from its 2002 crash (Chapter 3), I next established that while reproductive success appeared unaffected by changes in population density in the ranges I studied, at higher population densities individuals had significantly higher H/L ratios and heterophil counts, both of which are suggestive of higher levels of physiological stress. Saddlebacks in the recovered population also had lower levels of circulating lymphocytes and higher loads of feather mites, and this suggested a degree of immunosuppression at high population density, possibly brought about by the increased levels of stress. Thus, I identified two factors that

are possible key facilitators of disease outbreaks: susceptible hosts (stressed and/or immunodeficient) and high population densities. This has implications in terms of habitat size requirements, particularly regarding New Zealand birds where many endangered species are confined to islands because they cannot coexist with introduced mammalian predators on the mainland. Hence many of these populations are not only inbred as a consequence of severe bottlenecks, but also at high densities, often with a large number of different bird species competing for a limited space. It is concerning that many of these offshore island sanctuaries may in fact be providing the very conditions, such as high density and low genetic diversity, which could facilitate future disease outbreaks.

I next followed 12 bottlenecked populations of saddleback (Chapter 4), comparing levels of fluctuating asymmetry (FA) in three morphological traits (tarsus, nares and wattles), blood parameters, and parasite loads to determine if populations founded by fewer individuals have higher levels of stress and lower immunocompetence. In concurrence with several studies (e.g. Fowler & Whitlock 1994; Pomiankowski 1997; Gilligan *et al.* 2000; Kruuk *et al.* 2003), I found no relationship between bottleneck size and either tarsus, nares or wattle asymmetry in saddlebacks. However, I did find more bottlenecked populations had higher H/L ratios and feather mite loads and lower lymphocyte counts, which again highlighted immunodeficiency and higher levels of physiological stress in more bottlenecked populations. Additionally, I found that populations from smaller islands had higher H/L ratios and heterophil numbers, both associated with increased corticosterone levels, further highlighting high population densities as a key contributor to increased levels of stress as outlined in Chapter 3. I suggested the relationship between feather mite loads and founder number could be due to some underlying factor I did not measure that directly

affects feather mite persistence. One such variable I suggested was bill morphology, since it is the key tool in controlling ectoparasites, it would make sense that any developmental abnormality of the bill, possibly due to low levels of heterozygosity brought about by severe bottlenecks, would affect feather mite loads. Further work is required to verify this suggestion and thus far this is a relatively new angle in the field of parasitology (Clayton 1991; Clayton *et al.* 1999) .

One interesting finding of chapter 4 was the relationship between hippoboscids fly and coccidia parasite loads and tarsus FA. What was most intriguing was that tarsus FA increased with decreasing coccidia and increasing hippoboscids loads. This suggests that resistance to coccidia, which would involve the acquired immune system and production of antibodies, may result in a trade-off between development and immune function. In other words, a greater level of resistance may require more resources to be directed towards the immune system, perhaps at the cost of developmental homeostasis. Similarly, the cost of hosting hippoboscids flies is likely to increase as the number of flies increases, thus requiring greater investment into the immune system and diversion of resources away from development. The literature suggests three hormones (growth hormone, prolactin and thyroid hormone) are involved in both development and immune function (Apanius 1998), and may be the key factors involved in the trade-off between immune function and development. Further work investigating the effect of inbreeding on the endocrine system is certainly warranted.

Finally, in chapter 5 I examined the effect of population bottlenecks on wattle size, a sexually selected trait present in both male and female saddlebacks. Only recently has genetic heterozygosity and inbreeding been incorporated into traditional ‘good genes’

models of sexual selection and mate choice. As fitness often declines with homozygosity and inbreeding, females are expected to maximise offspring heterozygosity and fitness by choosing mates with a greater degree of genetic diversity or dissimilarity (Brown 1997). Males are therefore expected to advertise this genetic advantage through visual or behavioural displays (Brown 1997; Reid *et al.* 2005). So far there have been links made between the level of heterozygosity and behavioural sexual traits such as song repertoire and courtship (Meffert 1995; Aspi 2000; van Oosterhout *et al.* 2003; Reid *et al.* 2005), but not morphological traits such as plumage colour (Mariette *et al.* 2006) and no study looking at effects of inbreeding on sexual traits has considered female sexual ornaments. My findings indicated no effect of bottlenecks on wattle length for either males or females. Instead, I found a relationship between feather mite load and wattle length. However as in chapter 4, I suggest this relationship may be due to another underlying effect which I did not analyse. For example a trade-off may exist between preening behaviour and foraging: lower quality individuals, possibly with poorer territories in terms of resources, may be required to spend more time foraging at the expense of time spent preening. Such individuals are unlikely to be able to produce large wattles since doing so requires more carotenoids than can be obtained from the diet. In other words, carotenoids invested into sexual ornaments must be obtained from stored sources that would otherwise be used for other functions such as immune response. Thus large wattles together with low feather mite loads may indicate a high quality individual that has large enough carotenoid stores that it is able to both invest costly carotenoids into sexual ornaments and spend more time preening. At present my results are only correlative, but it would be worthwhile undertaking some experimental work that either reduces the stress on males and females

(e.g. food and carotenoid supplementation experiments) or increases it (e.g. inoculation with a parasite or pathogen). The importance of the wattles in mate choice also needs to be further tested, and whether the reduction in wattle size of saddlebacks I observed on some islands may be affecting patterns of mate choice and reproductive investment that in turn limit population growth.

One of the most striking findings in chapter 5 was the positive relationship between lymphocyte counts and wattle length in female saddlebacks. This has significance in terms of the influence the female has in protecting her offspring from diseases during early ontogeny and hence offspring survival and fitness. My results indicated that females with larger wattles have greater immunocompetence and therefore may have the ability to produce and transfer a larger quantity and diversity of antibodies (specifically immunoglobulins) to the offspring ensuring greater protection of the young bird to the array of novel pathogens it encounters upon hatching. This early aid may also allow for a greater growth given that more immune input from the mother may mean more resources can be directed towards growth. The role of maternal antibodies in early neonatal immune function has long been established and thus it is surprising that it has not been integrated in theories of sexual selection previously. It would appear that current theories of sexual selection are based on the assumption that offspring only benefit and inherit ‘good genes’ for immunocompetence from the father and while there are studies that demonstrate males with larger ornaments have a higher degree of immunocompetence, few have shown this to be directly linked to offspring immune function (Kleven *et al.* 2006; Kurtz & Sauer 1999). Sexual ornaments are common in females of some bird groups (e.g. parrots, auks) and based on this maternal role in offspring immunity, a male should maximise the survival and

fitness of his offspring by choosing a female with larger ornament which would signal superior immunocompetence and ability to transfer a greater quality and diversity of immunoglobulins to the offspring. Further work, such as comparing antibody levels between the mother, egg and chick and also cell-mediated and humoral immune responses in both sexes and the offspring, will be needed to gain a greater understanding of the function of sexual ornaments in both sexes and the specific role each sex plays in offspring fitness.

My research has focused on the complex nature of population bottlenecks and the mechanisms by which the subsequent loss of genetic diversity can influence the health of populations and their susceptibility to disease. I provide strong evidence indicating that severe population bottlenecks (and the loss of genetic diversity in general) increases a population's susceptibility to disease by disrupting the development and functioning of the immune system. This disruption appears to also enhance the cost of trade-offs between the immune system and other functions such as growth and reproduction. Thus, not only is the immediate health of the individual threatened but also potentially its lifetime fitness. What remains uncertain is the founder number threshold below which immune function is jeopardised. Although, based on the evidence illustrated in chapter 4 (figures 4.2 and 4.4) I did find evidence suggesting a threshold of around 90 – 110 individuals exists. Below this threshold the detrimental effects resulting from the loss of genetic diversity are relatively high in terms of immune function, but they appear to be similar whether you use 10 individuals or 50 individuals. However, there did appear to be a significant improvement when greater than 90 individuals are used. This suggests that conservation managers should aim to use at least 90 individuals to found new populations in order to minimise the

deleterious effects of bottlenecks on immune function. But it is also important to bear in mind that it may not be as simple as picking a number, particularly if the maintenance of MHC diversity depends upon individuals chosen. Even if sub-populations of endangered species do not differ significantly in overall levels of genetic diversity, they may differ in allelic diversity at the MHC loci. Because the acquired immune system of an individual depends to a large degree on what pathogens it has been exposed to, there may be substantial benefits from selecting subsets of individuals from different populations, given that these populations are likely to have been exposed to different parasites and diseases. This may be a critical factor in maintaining and possibly enhancing immunocompetence of new populations and certainly warrants further investigation.

In my thesis, I have touched upon some other factors which may act in synergy with inbreeding to further increase the effects of inbreeding and disease susceptibility of bottlenecked populations. Two key variables I identified were high population densities and small habitats. Here the findings tended to hinge around elevated stress levels and the immunosuppressive effect of this stress. Obviously not all inbred and endangered species will succumb to disease; however enhanced stress levels due to crowding and unsuitable sized habitats will increase the chances that such populations will be hit hardest should a disease be introduced. Thus it is not only genetic factors that must be taken into consideration when conserving endangered species; there is the question of habitat size, location and suitability, remembering that not all habitats in a given area will be suitable for any one particular species.

My results have a number of implications regarding conservation management in New Zealand, where species translocation is the key management tool and has resulted in

much of the endangered bird life being confined to isolated offshore islands largely due to an inability to coexist with mammalian predators on the mainland. While the translocation of wildlife has been, and is likely remain, a very successful conservation management tool worldwide and has undoubtedly saved many species from extinction, the method does invariably carry a high risk of introducing novel pathogens to endangered populations. Many of these translocations, both in New Zealand and around the world, have not considered disease and few have involved thorough health checks of individuals by experienced biologists or veterinarians (Griffith *et al.* 1993). As a result, numerous wildlife translocations have resulted in the introduction of diseases into endangered populations (examples given in Woodford 1993) and subsequently epidemics caused by wildlife translocations have decimated both free-ranging and captive populations in the past (reviewed by Cleaveland *et al.* 2002). Moreover, transferred individuals may become victims of diseases already existing in their new environment (Griffith *et al.* 1993). What is of particular concern with respect to New Zealand is that many of the islands used as refuges are relatively small on average, mainly owing to the logistical and funding constraints of removing mammalian predators from larger areas. Thus many of these populations are not only likely to be highly inbred but also to exist at high densities, enhancing not only stress levels but also disease transmission. Based on the findings of my research it is of concern that many of these offshore island sanctuaries may in fact be providing the very conditions required for a disease outbreak to occur.

Another factor increasing the likelihood of disease epidemics in endangered populations, and again one of particular concern to the New Zealand birdlife, is the presence of other more common bird species, particularly introduced species, which are

often present at relatively high densities and are able to move between reserves and outside areas. These species may be another key agent (besides translocation of wildlife) whereby disease is introduced into an endangered population (Lyles & Dobson 1993). This may be a particularly serious problem if a disease organism that could normally not persist once the endangered population declined below a specific threshold density, is maintained in the more common species (Lyles & Dobson 1993). In this situation, there is a high risk of diseases causing extinction of endangered populations. Indeed, it has been shown that most local extinctions and population crashes in endangered species follow this pattern (Cleaveland *et al.* 2002).

The process of detecting susceptible populations remains a difficult one. As is evident from my findings, haematological parameters provided the best measure of increased stress levels in saddlebacks and robins. However, for many populations of endangered species the baseline data simply does not exist to compare against in order to detect any deviations from 'normal' health, nor are there in many cases the resources to carry out large scale regular population health checks. This highlights the need to set up a monitoring programme which would enable managers to gather baseline data, facilitating faster detection of novel diseases and parasites. Prevention of the introduction of disease is of utmost importance and will require thorough disease screening prior to any movement of wildlife. Preventing elevated stress in populations (both genetic and environmental) will necessitate careful selection of habitat, taking into account the breeding behaviour of the species (i.e. how fast is it likely to reach carrying capacity of the island/habitat), habitat requirements (how much of the area is actually inhabitable by this species and overall area size), and the presence of all other species in the area. Selectively choosing individuals or

populations to found a new population should become a major factor in future translocation events and one that may provide a key to maximising the long-term viability of populations. Gaining further understanding of the relationship between inbreeding and immune function (including MHC) is critical if endangered species are to be appropriately managed and further declines or extinctions due to disease outbreaks are to be prevented. If genetic factors and disease are not adequately addressed there is a large risk of inappropriate management and failed restoration projects (Friend *et al.* 2001; Lyles & Dobson 1993).

6.1 References

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